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WDN:jam 11/09/01 4239-61380 77011

EXPRESS MAIL LABEL NO. EL874429598US
DATE OF DEPOSIT: November 9, 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER: 4239-61380

U S APPLICATION NO (If known, see 37 C F R § 1 5): 10/009210

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/12847	10 May 2000	12 May 1999

TITLE OF INVENTION
THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE

APPLICANT(S) FOR DO/EO/US
Nouri Neamati, Yves Pommier, Antonio Garofalo and Vito Nacci

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1 ☒ This is a **FIRST** submission of items concerning a filing under 35 U S.C § 371

2 ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U S C. § 371

3 ☒ This is an express request to begin national examination procedures (35 U.S C. § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U S C § 371(b) and PCT Articles 22 and 39(1)

4 ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date

5 ☐ A copy of the International Application as filed (35 U S.C. § 371(c)(2))

a ☐ is transmitted herewith (required only if not transmitted by the International Bureau)

b ☐ has been transmitted by the International Bureau

c ☒ is not required, as the application was filed in the United States Receiving Office (RO/US)

6 ☐ A translation of the International Application into English (35 U S C § 371(c)(2))

7 ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U S C § 371(c)(3))

a ☐ are transmitted herewith (required only if not transmitted by the International Bureau)

b ☐ have been transmitted by the International Bureau

c ☐ have not been made, however, the time limit for making such amendments has NOT expired

d ☒ have not been made and will not be made

8 ☐ A translation of the amendments to the claims under PCT Article 19 (35 U S C § 371(c)(3))

9 ☒ An oath or declaration of the inventor(s) (35 U.S.C. § 371(c)(4)).

10 ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S C § 371(c)(5))

Items 11. to 16. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 C F R. §§ 1 97 and 1.98

12 ☒ An assignment document for recording. A separate cover sheet in compliance with 37 C F R. §§ 3 28 and 3 31 and the Recordal fee of $40 00 is included

13. ☐ A **FIRST** preliminary amendment

☐ A **SECOND** or **SUBSEQUENT** preliminary amendment

14 ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter

16 ☒ Other items or information.

☐ Written Opinion

☐ Preliminary Examination Report

☐ International Search Report

☒ Copies of References Cited (in the Information Disclosure Statement).

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WDN:jam 11/09/01 4239-61380 77011

EXPRESS MAIL LABEL NO. EL874429598US
DATE OF DEPOSIT: November 9, 2001

U.S. APPLICATION NO (If known, see 37 C F R. § 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/009210	PCT/US00/12847	4239-61380

17. ☒ The following fees are submitted:

	CALCULATIONS (PTO USE ONLY)	
BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):		
Neither International Preliminary Examination fee (37 C F R. §1.482) nor International Search fee (37 C.F.R. § 1 445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO..... **$1,040.00**		
International Preliminary Examination fee (37 C F R § 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
International Preliminary Examination fee (37 C F R. § 1.482) not paid to USPTO but International Search fee (37 C.F.R § 1.445(a)(2)) paid to USPTO as an International Searching Authority. **$740.00**		
International Preliminary Examination fee paid to USPTO (37 C.F R § 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International Preliminary Examination fee paid to USPTO (37 C.F R § 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)..... **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C F R. §1 492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	45 - 20 =	25	x $18.00	$ 450.00	
Independent Claims	5 - 3 =	2	x $84.00	$ 168.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1,508.00	
☐ Reduction of 1/2 for filing by small entity Small entity status is claimed for this application.				$	
SUBTOTAL =				$ 1,508.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C F.R. §§1 492(f)) +				$	
TOTAL NATIONAL FEE =				$ 1,508.00	
Fee for recording the enclosed assignment (37 C.F R § 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C F R. §§ 3 28, 3.31) **$40.00** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 1,548.00	
				REFUND →	$
				CHARGE →	$1,548.00

a. ☒ Checks in the amount of $1,508 00 and $40 00 to cover the above fees are enclosed.

b. ☐ Please charge my Deposit Account No ____ in the amount of $ ____ to cover the above fees. A duplicate copy of this sheet is enclosed

c. ☒ The Director is hereby authorized to charge any additional fees that may be required, or credit any overpayment, to Deposit Account No 02-4550. A duplicate copy of this sheet is enclosed.

d. ☒ Please return the enclosed postcard to confirm that the items listed above have been received

NOTE: Where an appropriate time limit under 37 C.F.R. § 1.494 or § 1.495 has not been met, a petition to revive (37 C.F.R. § 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 S W. Salmon Street
Portland, OR 97204-2988

William D Noonan
SIGNATURE
William D. Noonan, M.D.
NAME
30,878
REGISTRATION NUMBER

cc: Docketing

10009210 , 110901

WDN:jam 11/09/01 4239-61380 77011

EXPRESS MAIL LABEL NO. EL874429598US
DATE OF DEPOSIT: November 9, 2001

FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371		4239-61380
		U S APPLICATION NO (If known, see 37 C F R § 1 5) 10/009210
INTERNATIONAL APPLICATION NO PCT/US00/12847	INTERNATIONAL FILING DATE 10 May 2000	PRIORITY DATE CLAIMED 12 May 1999
TITLE OF INVENTION THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE		
APPLICANT(S) FOR DO/EO/US Nouri Neamati, Yves Pommier, Antonio Garofalo and Vito Nacci		

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U S.C § 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U S.C. § 371
3. ☒ This is an express request to begin national examination procedures (35 U S C § 371(f) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S C § 371(b) and PCT Articles 22 and 39(1)
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   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
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13. ☐ A **FIRST** preliminary amendment
    ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    ☐ Written Opinion.
    ☐ Preliminary Examination Report
    ☐ International Search Report
    ☒ Copies of References Cited (in the Information Disclosure Statement)

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COPY

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EXPRESS MAIL LABEL NO. EL874429598US
DATE OF DEPOSIT: November 9, 2001

U.S. APPLICATION NO (If known, see 37 C.F.R. § 1.5) 10/009210	INTERNATIONAL APPLICATION NO PCT/US00/12847	ATTORNEY'S DOCKET NUMBER 4239-61380

17. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 C.F.R. §§ 1.492(a)(1)-(5)):

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	CALCULATIONS (PTO USE ONLY)	
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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
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MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 1,508.00	
☐ Reduction of 1/2 for filing by small entity Small entity status is claimed for this application				$	
SUBTOTAL =				$ 1,508.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 C.F.R. §§1.492(f)) +				$	
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TOTAL FEES ENCLOSED =				$ 1,548.00	
				REFUND →	$
				CHARGE →	$1,548.00

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SEND ALL CORRESPONDENCE TO

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, OR 97204-2988

William D Noonan
SIGNATURE
William D Noonan, M.D.
NAME
30,878
REGISTRATION NUMBER

cc: Docketing

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Neamati et al.

Application No. 10/009,210

Filed: November 9, 2001

For: THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE

Examiner: Not yet assigned

Date: January 8, 2002

Art Unit: Not yet assigned

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service on January 8, 2002 as First Class Mail in an envelope addressed to: BOX PCT, COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231.

William D Noonan

William D. Noonan, M.D.
Attorney for Applicant

BOX PCT
COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

PRELIMINARY AMENDMENT

Prior to examination of the above-referenced application, please amend the application as follows:

In the specification:

On page 1 after the title, please insert the following paragraph:

--PRIORITY CLAIM

This is a § 371 U.S. national stage of PCT/US00/12847 filed May 10, 2000, which was published in English under PCT Article 21(2), which in turn claims the benefit of U.S. Provisional Application 60/133,726 filed May 12, 1999.--

REMARKS

This Preliminary Amendment is submitted to recite the priority claim from corresponding International Application No. PCT/US00/12847 filed May 10, 2000, which claims the benefit of priority from U.S. Provisional Patent Application No. 60/133,726 filed May 12, 1999. No new matter is added. Entry of this amendment is respectfully requested.

The priority claim was already of record in the PCT application, where it was prominently noted in the PCT Request, and on the face of the published PCT application.

CONCLUSION

If any minor matters remain to be discussed prior to examination, the Examiner is invited to contact the undersigned at the telephone number listed below.

Respectfully submitted,

KLARQUIST SPARKMAN, LLP

By William D. Noonan
William D. Noonan, M.D.
Registration No. 30,878

One World Trade Center, Suite 1600
121 S.W. Salmon Street
Portland, Oregon 97204
Telephone: (503) 226-7391
Facsimile: (503) 228-9446

Marked-up Version of Amended Specification
Pursuant to 37 C.F.R. §§ 1.121(b)-(c)

In the specification:

In the specification, on page 1 after the title, please add the following paragraph:

PRIORITY CLAIM

This is a § 371 U.S. national stage of PCT/US00/12847 filed May 10, 2000, which was published in English under PCT Article 21(2), which in turn claims the benefit of U.S. Provisional Application 60/133,726 filed May 12, 1999.

THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE

FIELD OF THE INVENTION

The present invention concerns anti-retroviral drugs, and particularly prophylactic and therapeutic treatments for infections with the human immunodeficiency virus (HIV).

BACKGROUND OF THE INVENTION

HIV is a retrovirus that causes immunosuppression in humans (HIV disease), and leads to a disease complex known as the acquired immunodeficiency syndrome (AIDS). HIV disease is characterized by progressive functional deterioration of the immune system. The treatment of HIV disease has been significantly advanced by the recognition that combining different drugs with specific activities against different biochemical functions of the virus can help reduce the rapid development of drug resistant viruses that were seen in response to single drug treatment. However, even with combined treatments, multi-drug resistant strains of the virus have emerged. There is therefore a continuing need for the development of new anti-retroviral drugs that act specifically at different steps of the viral infection and replication cycle.

The integrase (IN) enzyme is an example of such a specific target. This enzyme catalyzes the insertion by virally-encoded integrase of proviral DNA into the host cell genome, which is the mechanism by which HIV and other retroviruses are introduced into human T-lymphoid cells. For HIV-1, this process is mediated by a 32 kD virally encoded integrase, having conserved sequences in the HIV long terminal repeats (LTR)[1]. Following reverse-transcription in the cytoplasm of infected cells, integrase cleaves two nucleotides from each of the viral DNA ends which contain a highly conserved CA motif. The cleaved DNA migrates to the nucleus as a part of a large nucleoprotein complex, where the integrase catalyzes the insertion of viral DNA into a host chromosome by a direct transesterification reaction.

In vitro assays have previously been developed to identify integrase inhibitors,[2,3] and have permitted the discovery of diverse classes of drugs that inhibit integrase.[5,6] However, the drugs discovered by these assays have not been highly selective and potent inhibitors of the integrase enzyme. Many of these drugs have additionally been non-selective inhibitors of reverse transcriptase or HIV protease, which limits their usefulness in combination therapy directed to different specific steps of the retroviral life cycle.

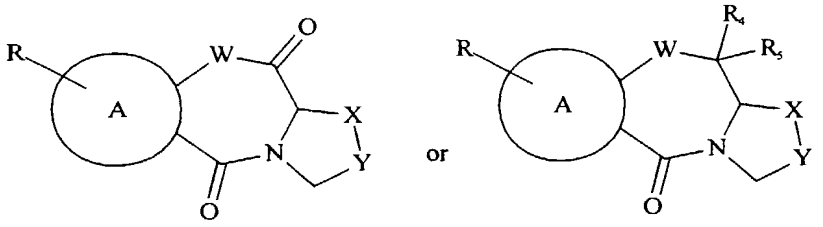
One class of reported integrase inhibitors is catechol-containing hydroxylated aromatics, which are non-selective integrase inhibitors that can also cross-link proteins[6] and chelate metals[7]. Non-catechol containing compounds, however, have been found to be cytotoxic, perhaps because they are unable to form reactive quinones. Such generalized cytotoxicity is a disadvantage, because it can affect host cells without being selective for retroviral eradication or inhibition.

Some hydrazides have been reported to be novel noncatechol-containing inhibitors of integrase. Structure-activity relationship studies among these inhibitors have indicated that the salicyl moiety is required for activity. Some benzothiazepine derivatives (such as diltiazem) have been reported to have lymphocyte protectant activity in U.S. Patent No. 4,861,770, and benzodiazepine hydrazide derivatives have been reported to inhibit HIV integrase in WO 98/18473. However, a specific anti-integrase inhibitor with minimal cytotoxic activity is not yet available.

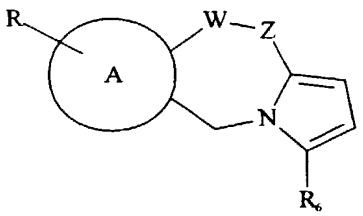
SUMMARY OF THE INVENTION

It has now surprisingly been found that certain noncatechol-containing compounds, including thiazepines, and particularly thiazolothiazepines, and analogs and derivatives thereof, are effective and selective anti-integrase inhibitors. The compounds of the present invention have particularly been found to inhibit both viral replication, and the activity of purified human immunodeficiency virus type-1 integrase (HIV-1 IN). Structure-activity studies have shown that the compounds possessing the pentatomic moiety SC(O)CNC(O) with two keto groups are in general more potent against purified IN than those containing only one keto group. Inhibitory potency against purified IN is maintained when the compounds are substituted with electron-donating or electron–withdrawing groups. Compounds with a naphthalene ring showed enhanced potency, as did those with thiazolo rings. Extension of the thiazole ring diminishes but did not eliminate potency.

The invention therefore includes a method of inhibiting a HIV integrase by exposing the integrase to an integrase inhibiting amount of one or more anti-integrase compounds, or pharmaceutically acceptable salts, of

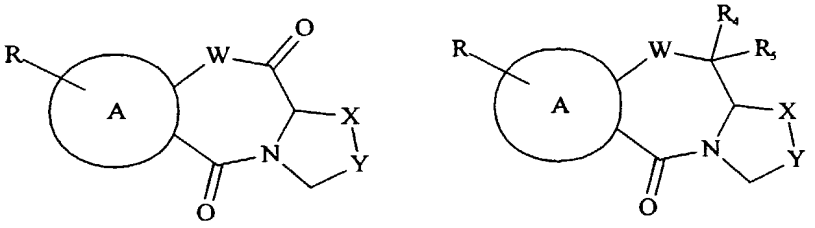


wherein A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; R is one or more of H, halogen, lower alkyl, lower alkoxy, NO_2, lower ester or carboxylic acid; X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2; R_4 is H or hydroxy; R_5 is H, phenyl, or alkylamine; and W is S or O or wherein the compound is



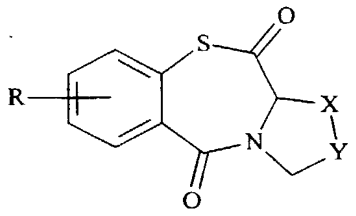
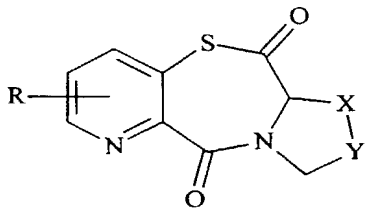
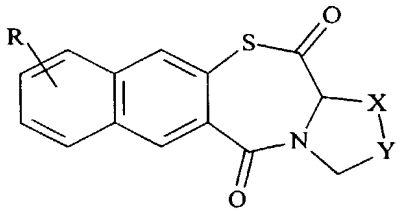
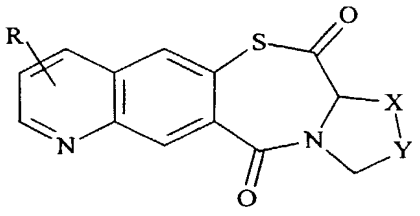
wherein A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; and R is one or more of H, halogen, lower alkyl, lower ester or carboxylic acid; R_6 is H, substituted or unsubstituted alkyl or amine; W is S or O; and Z is S, O, CH_2, CH_2CH_2, or C=O.

In yet other embodiments, the compound is selected from the group of

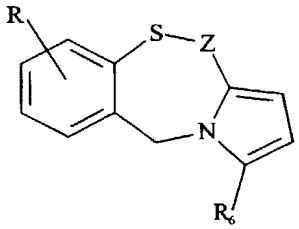


wherein X-Y is CH_2-S, S-CH_2, CH_2-O, or CH_2-CH_2, and W is S. In particular compounds, A is benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline, and in particular benzene or naphthalene. In other particular examples, R is H, halogen, lower alkoxy, or NO_2.

In another aspect of the invention, the compound is one or more of:

In yet other embodiments, the compound is one or more of: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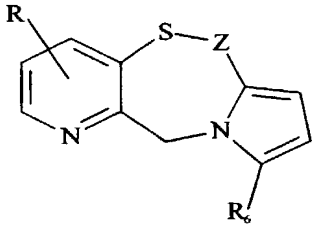
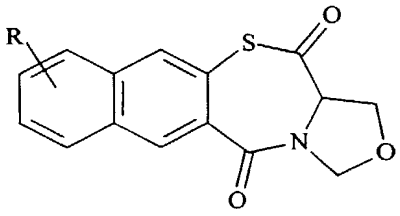
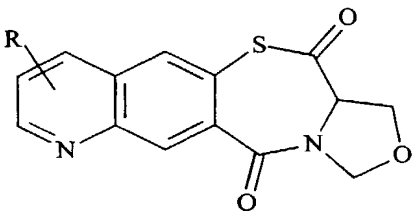
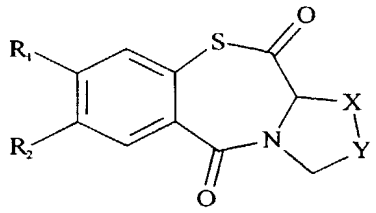
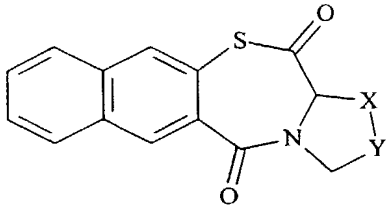
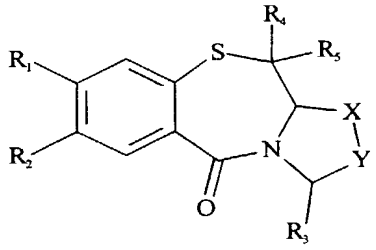
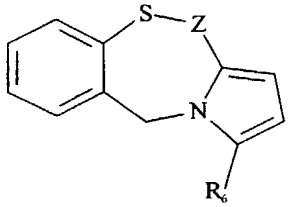


In more specific embodiments, the compound is one or more of:


or


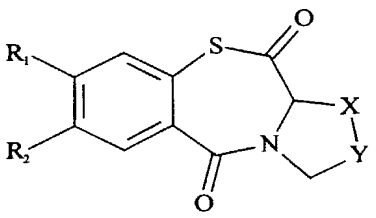
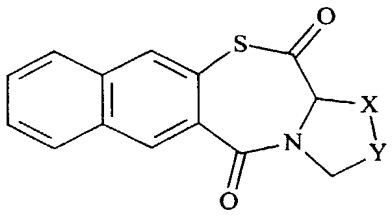
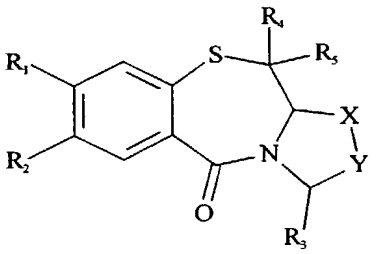
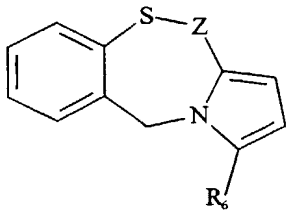
or




or


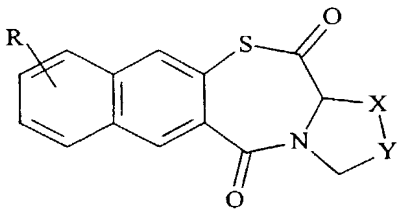
wherein X-Y is S-CH_2, CH_2-S, CH_2-O, CH_2-CH_2, S(O)-CH_2, or CH_2-S(O); R_1 and R_2 are independently selected from the group consisting of H, NO_2, halogen, lower alkyl or lower alkoxy; R_3 is H or phenyl; R_4 is H or hydroxy; R_5 is H, phenyl or alkylamine; and R_6 is H, phenyl or alkylamine.

In particular embodiments, the alkylamine is -$N(CH_2CH_2)_2NCH_3$, -$CH_2NCH_2CH_3$, or -$CH_2N(CH_2CH_2)_2NCH_3$.

A particular group of compounds with superior anti-integrase activity is

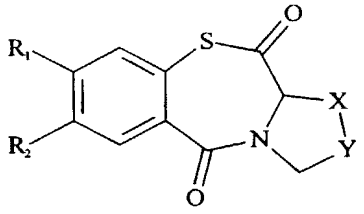



or


wherein X-Y is S-CH_2, CH_2-S, or CH_2-S(O); and R_1 and R_2 are independently selected from the group consisting of H, NO_2, halogen, lower alkyl and lower alkoxy; R_3 is H; and R_4, R_5, and R_6 are H. An example of such a compound is



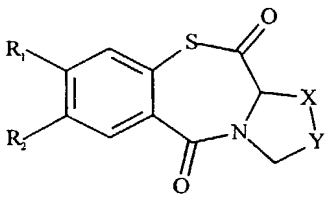
wherein X-Y is S-CH_2 or CH_2-S, and for example where R is H and X-Y is S-CH_2.

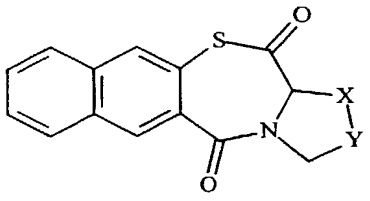
In other embodiments, in which the compounds have particularly good antiviral activity, R_1 is H, NO_2, or lower alkoxy; R_2 is H, Cl, Br, lower alkyl, or lower alkoxy; R_3 and R_4 are H; R_5 is $N(CH_2CH_2)_2NCH_3$; and X-Y is CH_2-S, S-CH_2, or CH_2-CH_2, and particularly:

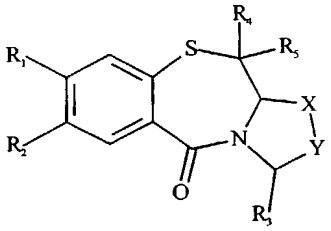


wherein R_1 is H, NO_2, or methoxy; R_2 is H, halogen or methoxy; and X-Y is CH_2-S or S-CH_2, especially S-CH_2.

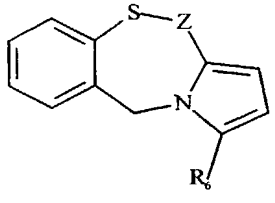
The invention also includes methods of administering any of the above compounds in a therapeutically effective amount to a subject, for example to treat or prevent HIV infection in the subject. In particular examples, the compound which is administered is selected from the group of



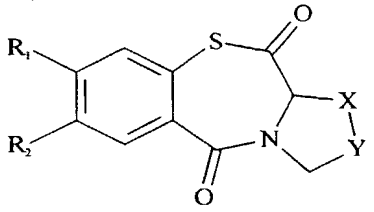




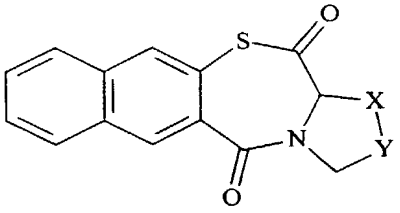
and



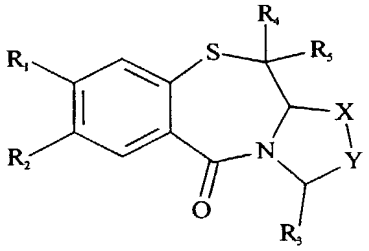
wherein X-Y is S-CH_2, CH_2-S, CH_2CH_2 or S(O)CH_2; R_1 is H, NO_2, or lower alkoxy; R_2 is H, Cl, Br, lower alkyl, or lower alkoxy; R_3 and R_4 are H; R_5 is $N(CH_2CH_2)_2NCH_3$; and R_6 is H, and in particular:



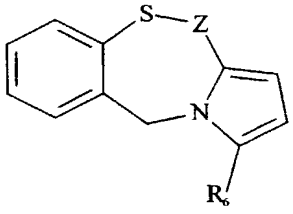
wherein R_1 and R_2 are H, and X-Y is S-CH_2; or R_1 is H, R_2 is Cl or Br or methyl, and X-Y is S-CH_2; or R_1 is NO_2, R_2 is H, and X-Y is CH_2-S; or R_1 and R_2 are methoxy, and X-Y is CH_2-S; or R_1 is H, R_2 is methyl, and X-Y is S(O)-CH_2; or



wherein X-Y is S-CH_2 or CH_2-S; or



wherein X-Y is CH_2-CH_2; R_1, R_2, R_3 and R_4 are H; and R_5 is $N(CH_2CH_2)_2NCH_3$; or

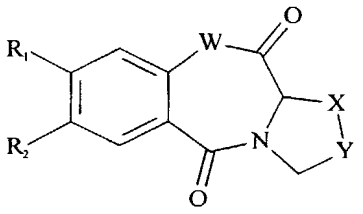


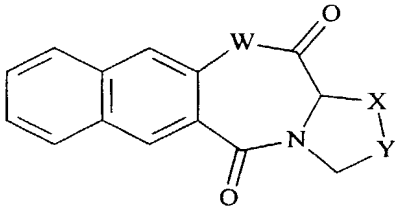
wherein R_6 is H and Z is C=O.

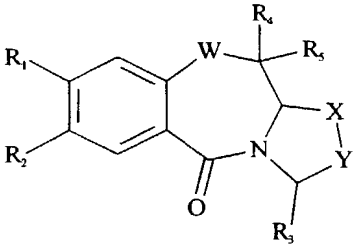
In other embodiments, R is one or more of halogen or NO_2; X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2; R_4 is H or hydroxy, particularly hydroxy; R_5 is H, phenyl, or alkylamine;

and W is S or O; R_6 is H, substituted or unsubstituted alkyl or amine; Z is S, O, CH_2, CH_2CH_2, or C=O, particularly C=O.

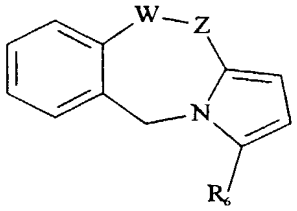
In particular embodiments, the method includes administration of:





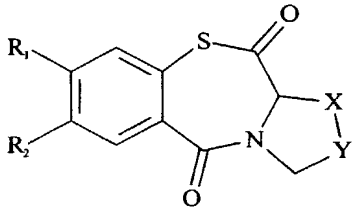


or



wherein X-Y is S-CH_2, CH_2-S, S(O)-CH_2, CH_2-S(O), or CH_2CH_2; W is S or O; R_1 is H or NO_2; R_2 is H, halogen, lower alkyl or lower alkoxy; R_3 is H; R_4 is hydroxy or H; R_5 is phenyl or $N(CH_2CH_2)_2NCH_3$; and R_6 is $CH_2N(CH_2CH_2)_2NCH_3$, provided that R_1 and R_2 are not both H or not both lower alkoxy (such as methoxy).

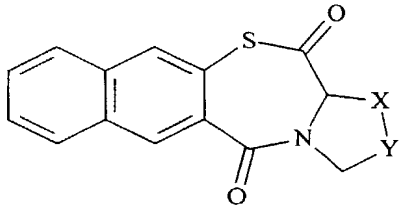
For example, the administered compound can be



wherein R_1 is H or NO_2; R_2 is H, halogen, lower alkyl or lower alkoxy; provided that R_1 and R_2 are not both H or lower alkoxy; or wherein R_1 is H, R_2 is Cl, X-Y is S-CH_2; or R_1 is H, R_2 is Br, X-Y is S-CH_2; or R_1 is H, R_2 is CH_3, X-Y is S-CH_2; or R_1 is H, R_2 is H, X-Y is CH_2-S; or R_1 is H, R_2 is Cl, X-Y is CH_2-S; or R_1 is H, R_2 is Br, X-Y is CH_2-S; or R_1 is H, R_2 is CH_3, X-Y is CH_2-S; or R_1 is NO_2, R_2 is H, X-Y is CH_2-S; or R_1 is H, R_2 is OCH_3, X-Y is CH_2-S; or R_1 is H, R_2 is H, X-Y is CH_2-O; or R_1 is H, R_2 is CH_3, X-Y is S(O)-CH_2; or R_1 is H, R_2 is H, X-Y is CH_2-S(O); or R_1 is H, R_2 is Cl, X-Y is CH_2-S(O); or R_1 is H, R_2 is OCH_3, X-Y is CH_2-S(O).

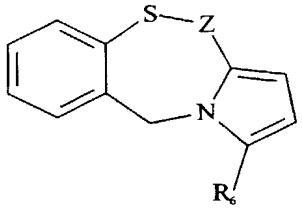
In particular, X-Y may be S-CH_2. Alternatively, R_1, R_2 and R_3 are H, R_4 is OH or H, R_5 is Ph or $N(CH_2CH_2)_2CH_3$, and X-Y is CH_2-CH_2.

Alternatively, the compound can be:



wherein X-Y is S-CH_2 or CH_2-S.

The compound can also be:



wherein R_6 is $CH_2N(CH_2CH_2)_2NCH_3$.

The invention also includes a pharmaceutical composition that includes the compound, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. Also included are methods of screening for an anti-HIV integrase drug, by providing an assay of HIV integrase inhibition, and using the assay to screen for drugs that are analogs or derivatives of any of the compounds, and which inhibit HIV integrase. In particular embodiments, the assay detects a thiazepine compound that inhibits human immunodeficiency virus type-1 integrase (HIV-1 IN), and particularly such a compound that has no detectable effect on reverse transcriptase, protease, and virus attachment. In very particular examples, the compounds that are screened are thiazolothiazepines.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a schematic diagram illustrating an assay for integrase mediated integration of HIV DNA into the host cell genome. A 21-mer blunt-end oligonucleotide corresponding to the U5 end of the HIV-1 LTR, 5' end-labeled with ^{32}P, is reacted with purified integrase. The enzyme causes nucleolytic cleavage of two bases from the 3'-end (3' processing), resulting in a 19-mer oligonucleotide. Subsequently, 3' ends are covalently joined to

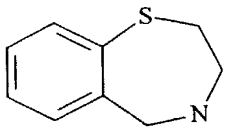
another identical oligonucleotide that serves as the target DNA (strand transfer reaction).

FIGS 2 and 3 show concentration dependent inhibition of HIV-1 IN by thiazolothiazepines **1, 19,** and **20** using Mn^{+2} (FIG. 2) and Mg^{+2} (FIG. 3) as a cofactor. Lane 1, DNA alone, lanes 2 and 15 DNA and integrase, lanes 3-6, 7-10, 11-14 DNA, integrase, and compounds **1**, **19**, and **20** at 1000, 333, 111, and 37 μM, respectively. See Table 1 for compound identifiers **1,19,20**.

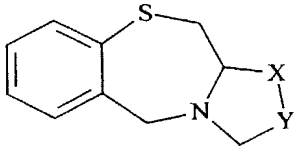
DETAILED DESCRIPTION OF PARTICULAR EXAMPLES

Definitions

A "benzothiazepine" refers to a benzodiazepine in which one of the ring nitrogens has been replaced with a sulfur, for example:



while a "thiazolothiazepine" refers to a benzothiazepine having a thiazolo ring, for example:



in which X-Y is CH_2-S or S-CH_2.

The term "alkyl" refers to a cyclic, branched, or straight chain alkyl group containing only carbon and hydrogen, and unless otherwise mentioned contains one to twelve carbon atoms. This term is further exemplified by groups such as methyl, ethyl, n-propyl, isobutyl, t-butyl, pentyl, pivalyl, heptyl, adamantyl, and cyclopentyl. Alkyl groups can either be unsubstituted or substituted with one or more substituents, e.g. halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "lower alkyl" refers to a cyclic, branched or straight chain monovalent alkyl radical of one to five carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, i-propyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), cyclopropylmethyl, i-amyl, and n-amyl. Lower alkyl

groups can also be unsubstituted or substituted, where a specific example of a substituted alkyl is 1,1-dimethyl propyl.

"Carboxyl" refers to the radical -COOH, and substituted carboxyl refers to -COR where R is alkyl, lower alkyl or a carboxylic acid or ester.

The term "aryl" refers to a monovalent unsaturated aromatic carbocyclic group having a single ring (e.g. phenyl) or multiple condensed rings (e.g. naphthyl or anthryl), which can optionally be unsubstituted or substituted with, e.g., halogen, alkyl, alkoxy, mercapto (-SH), alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "alkoxy" refers to a substituted or unsubstituted alkoxy, where an alkoxy has the structure -O-R, where R is substituted or unsubstituted alkyl. In an unsubstituted alkoxy, the R is an unsubstituted alkyl. The term "substituted alkoxy" refers to a group having the structure -O-R, where R is alkyl which is substituted with a non-interfering substituent. "Lower alkoxy" refers to any alkoxy in which R is a lower alkyl.

The term "heterocycle" refers to a monovalent saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g. benzyl, morpholino, pyridyl or furyl) or multiple condensed rings (e.g. naphthyl, quinolinyl, indolizinyl or benzo[b]thienyl) and having at least one heteroatom, defined as N, O, P, or S, within the ring, which can optionally be unsubstituted or substituted with, e.g. halogen, alkyl, alkoxy, alkylthio, trifluoromethyl, acyloxy, hydroxy, mercapto, carboxy, aryloxy, aryl, arylalkyl, heteroaryl, amino, alkylamino, dialkylamino, morpholino, piperidino, pyrrolidin-1-yl, piperazin-1-yl, or other functionality.

The term "halogen" refers to fluoro, bromo, chloro and iodo substituents.

The term "amino" refers to a chemical functionality $-NR_1R_2$ where R_1 and R_2 are independently hydrogen, alkyl, or aryl.

A "pharmaceutical agent" or "drug" refers to a chemical compound or composition capable of inducing a desired therapeutic or prophylactic effect when properly administered to a subject.

The pharmaceutically acceptable salts of the compounds of this invention include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methyl-glutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine,

tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide. These salts may be prepared by standard procedures, for example by reacting the free acid with a suitable organic or inorganic base. Any chemical compound recited in this specification may alternatively be administered as a pharmaceutically acceptable salt thereof.

All chemical compounds include both the (+) and (-) stereoisomers, as well as either the (+) or (-) stereoisomer.

An analog is a molecule, that differs in chemical structure from a parent compound, for example a homolog (differing by an increment in the chemical structure, such as a difference in the length of an alkyl chain), a molecular fragment, a structure that differs by one or more functional groups, or a change in ionization. Structural analogs are often found using quantitative structure activity relationships (QSAR), with techniques such as those disclosed in *Remington: The Science and Practice of Pharmacology*, 19th Edition (1995), chapter 28. A derivative is a biologically active molecule derived from the base structure. A mimetic is a biomolecule that mimics the activity of another biologically active molecule. Biologically active molecules can include both chemical structures and peptides of protein entities that mimic the biological activities of the mercaptosalicylhydrazides of the present invention.

Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (1985) and *The Condensed Chemical Dictionary* (1981).

A "mammal" includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

An animal is a living multicellular vertebrate organism, a category which includes, for example, mammals and birds.

"HIV disease" refers to a well recognized constellation of signs and symptoms (including the development of opportunistic infections) in persons who are infected by an HIV virus, as determined by antibody or western blot studies. Laboratory findings associated with this disease are a progressive decline in T-helper cells.

Materials and Methods

Where necessary, solvents were dried and purified according to the recommended procedures. All reactions were carried out under an argon atmosphere. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Organic solutions were dried over $MgSO_4$,

evaporation refers to removal of solvent on a rotary evaporator under reduced pressure. Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. Optical rotations were measured on an Optical Activity AA-5 polarimeter. IR spectra were recorded as thin films on Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Mass spectral data were determined by direct insertion at 70 eV with a VG70 spectrometer. Merck silica gel (Kieselgel 60/230-400 mesh) was used for flash chromatography columns. Dowex 50x2 200 resin (Aldrich) was used for ion-exchange chromatography. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within $\pm$0.4% of the theoretical values. Yields refer to purified products and are not optimized.

Compound Reference Numbers

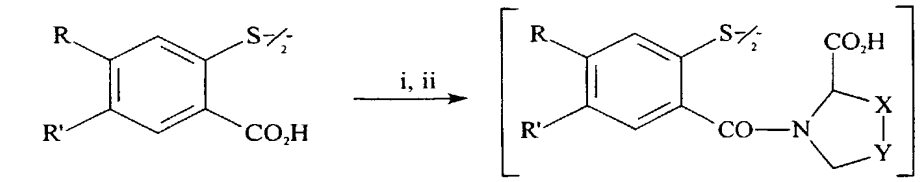
Compounds are identified throughout this detailed description using reference numerals in bold, which correspond to the identification of the compounds in Table 1, and in the following examples.

EXAMPLE 1

Synthesis of Thiazolothiazepines

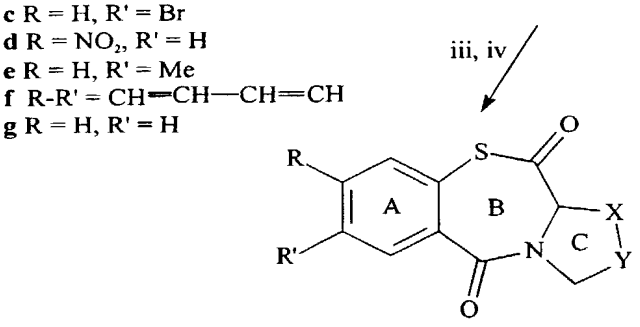
The synthesis of compounds **1**, **5**, **11**, **13-14**, **21-25** and **28-30** (see Table 1 for an identification of these compounds) has been accomplished according to general or similar procedures reported elsewhere.[15-20] Compounds **2-4**, **6-10**, **12** and **19-20** (Table 1) have been prepared following the general method outlined in Scheme 1:

Scheme 1

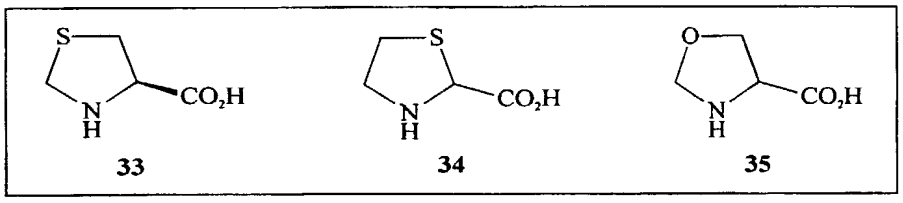


32a R = H, R' = OMe
b R = H, R' = Cl
c R = H, R' = Br
d R = NO_2, R' = H
e R = H, R' = Me
f R-R' = CH=CH—CH=CH
g R = H, R' = H

36a-g



1-14, 19-20



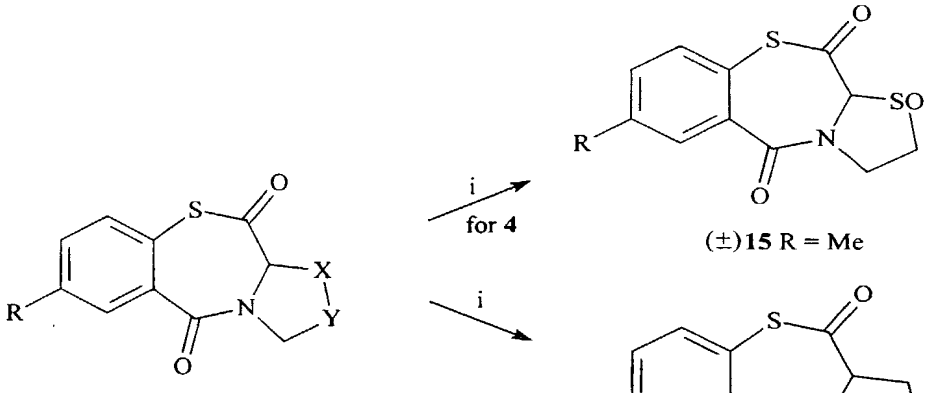
Reagents: (i) $SOCl_2$/reflux; (ii) 33 or 34 or 35 /Na_2CO_3/THF/H_2O/rt; (iii) $NaBH_4$/EtOH/reflux; (iv) CDI/THF/reflux.

Schotten-Baumann reaction between the (un)substituted 2,2'-dithiobis(benzoic acid chloride) or 3,3'-dithiobis(2,2'-naphtoic acid chloride), in turn obtained from corresponding acids **32a-g** [21-25] and thionyl chloride, and L-thiaproline (Aldrich) **(33)** or thiazolidine-2-carboxylic acid **(34)** or 1,3-oxazolidine-4-carboxylic acid **(35)** (Chart 1) gave disulfides **36**. $NaBH_4$ reduction of the crude disulfides gave the corresponding thiophenols in very good yield. Disulfides **36** and subsequent thiophenols were obtained as amorphous solids by ion-exchange chromatography and were then used without a thorough characterization. The eventual cyclization reaction was carried out using N,N'-carbonyldiimidazole (CDI) in dry THF leading to optically inactive tricyclics **2-4**, **6-10**, **12** and **19-20** because of racemization at C-11a (C-13a in the case of compounds **19** and **20**).

Controlled 3-chloroperbenzoic acid (MCPBA) oxidation of the tricyclic sulfides **4-6** and **10** gave sulfoxides **15-18** (Scheme 2) which were tentatively

assigned a *cis* configuration on the basis of ^{1}H NMR experiments and Dreiding stereomodels inspection. This would reflect a preferred approach of the electrophile from the less hindered face of the tricyclic ring, namely the one taken up by the C-.11a proton. Example 4 provides more details about synthesis of the sulfoxides 15-18.

Scheme 2



(±)**4** R = Me, X-Y = SCH_2
(±)**5** R = H, X-Y = CH_2S
(±)**6** R = Cl, X-Y = CH_2S
(±)**10** R = OMe, X-Y = CH_2S

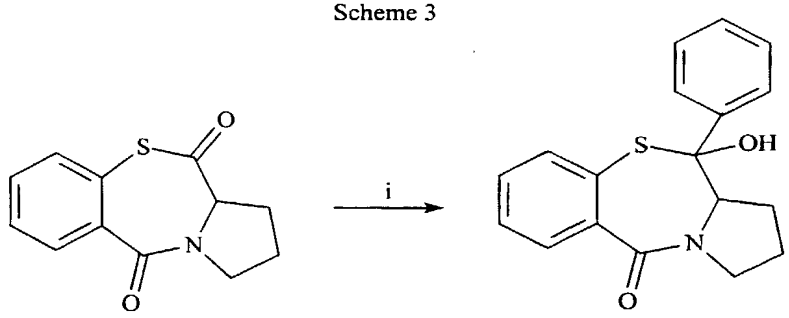
(±)**15** R = Me

(±)**16** R = H
(±)**17** R = Cl
(±)**18** R = OMe

Reagents: (i) MCPBA/CH_2Cl_2/0 °C

Compound **26** was obtained as a racemic mixture by reaction of previously described (±)-1,2,3,11a-tetrahydro-5*H*,11*H*-pyrrolo[2,1-*c*][1,4]benzothiazepine-5,11-dione **(37)** with phenyl magnesium bromide in dry conditions (Scheme 3).

Scheme 3



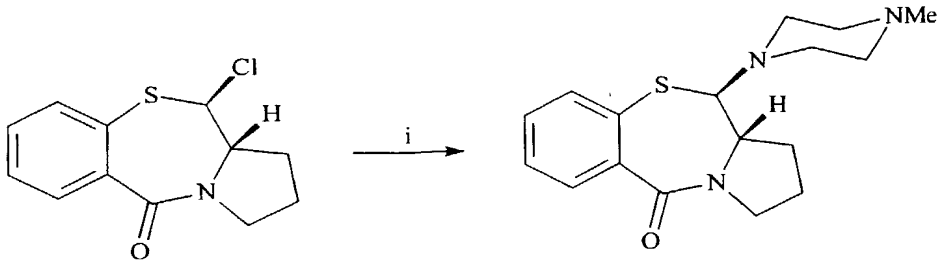
(±) **37**

(±) **26**

Reagents: (i) PhMgBr/Et_2O.

The basic side chain of compound **27** was installed by means of reaction of 1-methylpiperazine on optically active chloro derivative **38** (Scheme 4).

Scheme 4

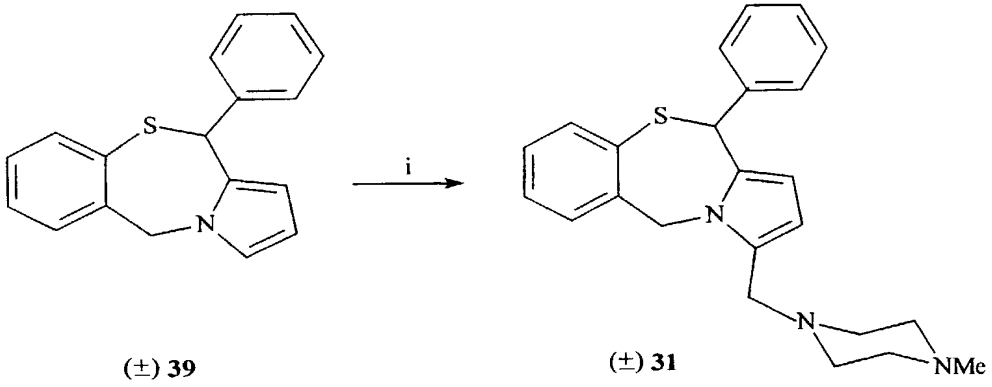


(±) **38** (±) **27**

Reagents: (i) $HN(CH_2CH_2)_2NMe/Na_2CO_3/NaI/MeCN/reflux/20$ h.

In the reaction conditions used, a racemic mixture of *cis* **27** was the sole product obtained. Finally, the synthesis of compound **31** was carried out by Mannich reaction on the α-pyrrole position of (±)-11-phenyl-5*H*,11*H*-pyrrolo[2,1-c][1,4]benzothiazepine **(39)** by means of paraformaldehyde and 1-methylpiperazine dihydrochloride in methanol (Scheme 5).

Scheme 5



(±) **39** (±) **31**

Reagents: (i) $CH_2O/HN(CH_2CH_2)_2NMe.2HCl/MeOH.reflux$ 48h.

EXAMPLE 2

Procedure for the Preparation of Thiazepine 10

This example illustrates the preparation of (±)-1,11a-dihydro-7-methoxy-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4]benzothiazepine-5,11-dione **(10)**. A mixture of

2,2'-dithiobis(5-methoxybenzoic acid) (5.5 g, 15 mmol) **(32a)** and thionyl chloride (40 mL) was refluxed for 1.5 hours. After cooling, the excess of thionyl chloride was removed under vacuum using dry benzene (2 x 5 mL). The resulting solid was dissolved in dry THF (40 mL) and the solution was added dropwise to a mixture of L-thiaproline **(33)** (4.0 g, 30.0 mmol) and sodium carbonate (3.2 g, 15.0 mmol) in water (50 mL). Additional sodium carbonate was added from time to time to maintain a weakly alkaline pH. The mixture was stirred overnight, then concentrated and made acidic (pH 3-4) by adding concentrated HCl. The gummy solid was extracted into ethyl acetate and the resulting solution was washed with water, dried, and evaporated to give foam. Ion-exchange column chromatography using methanol as the eluent gave almost pure disulfide **36a** as an amorphous solid.

The solid (8.95 g, 15 mmol) was dissolved in 85% ethanol (100 mL) containing NaOH (1.2 g, 30 mmol), and to this was added a solution of $NaBH_4$ (1.14 g, 30 mmol) in ethanol (50 mL). The mixture was gently refluxed for 0.5 hour, then concentrated and diluted with chilled-water. The cold solution was left for 15 minutes before being filtered and made acidic (pH 3-4) by concentrated HCl. The gummy solid was treated exactly as described before for purification of compound **36a** and then thoroughly dried under vacuum before being subjected to the successive reaction without further manipulation. Crude thiophenol derivative (6.0 g, 20.0 mmol) was dissolved into dry THF (80 mL) and N,N'-carbonyldiimidazole (3.24 g, 20.0 mmol) was added in portions. The solution was stirred for 24 hours, and then for 2 hours under reflux. The solvent was evaporated and the residue was partitioned between $CHCl_3$ and 0.5 N HCl. The organic solution was separated and washed with $NaHCO_3$ saturated solution and water.

After drying and evaporation of the solvent, a pasty residue was obtained and purified by flash chromatography (8% methanol in EtOAc) to give 4.2 g (74% yield from the thiophenol precursor) of **10** as a white powder. Analysis revealed mp 145-147 °C (benzene); IR (KBr) 1700, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.46 (d, 1 H, J = 2.8 Hz), 7.35 (m, 1 H), 7.04 (dd, 1 H, J = 8.1, 2.8 Hz), 4.79 (AB q, 2 H, J = 10.2 Hz), 4.60 (dd, 1 H, J = 6.6, 1.5 Hz), 3.87 (s, 3 H), 3.64 (dd, 1 H, J = 12.7, 1.5 Hz), 3.15 (dd, 1 H, J = 12.7, 6.6 Hz). Anal. ($C_{12}H_{11}NO_3S_2$) C, H, N.

EXAMPLE 3
Synthesis and Analytical Results for Compounds 2-4, 6-9, 12, 19-20, 26-27, and 31

(±)-7-Chloro-2,3-dihydro-5*H*-thiazolo[2,3-*c*][1,4]benzothiazepine-5,11 (11a*H*)-dione (2). Starting from 2,2'-dithiobis(5-chlorobenzoic acid) **(32b)** (5.6 g, 15 mmol), the title compound **2** was obtained (3.6 g, 63% yield from the thiophenol precursor) adopting the same procedure as for **10**, but using thiazolidine-2-carboxylic acid **34** instead of L-thiaproline **33**: mp 231-232 °C (benzene); IR (KBr) 1695, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.98 (d, 1 H, J = 2.4 Hz), 7.52-7.38 (m, 2 H), 5.35 (s, 1 H), 4.03 (m, 2 H), 3.16 (m, 2 H). Anal. ($C_{11}H_8ClNO_2S_2$) C, H, N.

(±)-7-Bromo-2,3-dihydro-5*H*-thiazolo[2,3-*c*][1,4]benzothiazepine-5,11 (11a*H*)-dione (3). Starting from 2,2'-dithiobis(5-bromobenzoic acid) **(32c)** [22] (7.0 g, 15 mmol), the compound **3** was obtained (4.0 g, 70% yield from the thiophenol precursor) adopting the same procedure as for **10**, but using thiazolidine-2-carboxylic acid **34** instead of L-thiaproline **33**: mp 219-220 °C (benzene); IR (KBr) 1695, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.11 (d, 1 H, J = 2.2 Hz), 7.62 (dd, 1 H, J = 8.3, 2.2 Hz), 7.32 (dd, 1 H, J = 8.3, 2.2 Hz), 5.33 (s, 1 H), 4.00 (m, 2 H), 3.18 (m, 2 H). Anal. ($C_{11}H_8BrNO_2S_2$) C, H, N.

(±)-2,3-Dihydro-7-methyl-5*H*-thiazolo[2,3-*c*][1,4]benzothiazepine-5,11 (11a*H*)-dione (4). Starting from 2,2'-dithiobis(5-methylbenzoic acid) **(32e)** (5.0 g, 15.0 mmol), the compound **4** was obtained (5.6 g, 76% yield from the thiophenol precursor) adopting the same procedure as for **10**, but using thiazolidine-2-carboxylic acid **34** instead of L-thiaproline **33**: mp 197-199 °C (benzene - petroleum ether); IR (KBr) 1690, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.79 (s, 1 H,), 7.29 (m, 2 H), 5.36 (s, 1 H), 4.02 (m, 2 H), 3.12 (m, 2 H), 2.42 (s, 3 H). Anal. ($C_{12}H_{11}NO_2S_2$) C, H, N.

(±)-7-Chloro-1,11a-dihydro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione (6). Starting from 2,2'-dithiobis(5-chlorobenzoic acid) **(32b)** (5.6 g, 15 mmol), the compound **7** was obtained (4.0 g, 70% yield from the thiophenol precursor) using the same procedure as for **10**: mp 204-205 °C (benzene). IR (KBr) 1705, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.96 (d, 1 H, J = 2.0), 7.51-7.38 (m, 2 H), 4.80 (AB q, 2 H, J = 10.7 Hz), 4.57 (dd, 1 H, J =

6.9, 1.7 Hz), 3.67 (dd, 1 H, J = 11.9, 1.7 Hz), 3.19 (dd, 1 H, J = 11.9, 6.9 Hz). Anal. ($C_{11}H_8ClNO_2S_2$) C, H, N.

(±)-7-Bromo-1,11a-dihydro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione (7). Starting from 2,2'-dithiobis(5-bromobenzoic acid) **(32c)** (7.0 g, 15 mmol), the compound **8** was obtained (4.1 g, 63% yield from the thiophenol precursor) using the same procedure as for **10**: mp 213-214 °C (benzene). IR (KBr) 1700, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.10 (t, 1 H, J = 1.0 Hz), 7.63 (dd, 1 H, J = 8.0, 1.0), 7.34 (d, 1 H, J = 8.0), 4.81 (AB q, 2 H, J = 10.6 Hz), 4.55 (dd, 1 H, J = 6.0, 1.9 Hz), 3.67 (dd, 1 H, J = 11.8, 1.9 Hz), 3.18 (dd, 1 H, J = 11.8, 6.0 Hz). Anal. ($C_{11}H_8BrNO_2S_2$) C, H, N.

(±)-1,11a-Dihydro-7-methyl-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione (8). Starting from 2,2'-dithiobis(5-methylbenzoic acid) **(32e)** (5.0 g, 15.0 mmol), the compound **8** (6.1 g, 82% yield from the thiophenol precursor) was obtained as a thick oil, using the same procedure as for **10**: IR (KBr) 1695, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.74 (s, 1 H,), 7.30 (m, 2 H), 4.77 (AB q, 2 H, J = 10.2 Hz), 4.60 (dd, 1 H, J = 6.8, 1.7 Hz), 3.62 (dd, 1 H, J = 12.1, 1.7 Hz), 3.23 (dd, 1 H, J = 12.1, 6.8 Hz), 2.40 (s, 3 H). Anal. ($C_{12}H_{11}NO_2S_2$) C, H, N.

(±)-1,11a-Dihydro-8-nitro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione (9). Starting from 2,2'-dithiobis(4-nitrobenzoic acid) **(32d)** (5.9 g, 15 mmol), the compound **9** was obtained (2.0 g, 34% from the thiophenol precursor) using the same procedure as for **10**: mp 231-232 °C (benzene - petroleum ether); IR (KBr) 1715, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.35 (s, 1 H,), 8.32 (dd, 1 H, J = 8.1, 2.0 Hz), 8.16 (d, 1 H, J = 8.1 Hz), 4.84 (AB q, 2 H, J = 10.5 Hz), 4.54 (dd, 1 H, J = 6.4, 1.5 Hz), 3.70 (dd, 1 H, J = 11.9, 1.5 Hz), 3.23 (dd, 1 H, J = 11.9, 6.4 Hz). Anal. ($C_{11}H_8N_2O_4S_2$) C, H, N.

(±)-1,11a-Dihydro-3*H*,5*H*,11*H*-oxazolo[4,3-*c*][1,4]benzothiazepine-5,11-dione (12). Starting from commercial 2,2'-dithiodibenzoic acid **(32g)** (4.6 g, 15.0 mmol), the compound **12** was obtained as a thick oil (3.0 g, 42% yield from the thiophenol precursor) adopting the same procedure as for **10**, but using freshly prepared 1,3-oxazolidine-4-carboxylic acid **32** [27] (3.5 g, 30.0 mmol) instead of L-thiaproline **33**: IR (KBr) 1690, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.50 (m, 1 H,),

7.22 (m, 3H), 5.22 (AB q, 2 H, J = 5.3 Hz), 4.81 (dd, 1 H, J = 8.8, 1.7 Hz), 4.25 (dd, 1 H, J = 5.9, 1.6 Hz), 3.98 (dd, 1 H, J = 8.9, 5.9 Hz). MS *m/z* 235 (M^+), 207, 177, 150, 136 (100), 108. Anal. ($C_{11}H_9NO_3S$) C, H, N.

(±)-2,3-Dihydro-5*H*-naphto[2,3-*f*]thiazolo[2,3-*c*][1,4]thiazepine-5,13(13a*H*)-dione (19). Starting from 3,3'-dithiobis(2,2'-naphtoic acid) **(32f)** (1.22 g, 1.5 mmol), the compound **19** (0.27 g, 30% yield from the thiophenol precursor) was obtained, adopting the same procedure as for **10**, but using thiazolidine-2-carboxylic acid **34** instead of L-thiaproline **33** and carrying out the $NaBH_4$ reduction of the disulfide overnight at room temperature: mp 196-198 °C; IR (KBr) 1700, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.52 (s, 1 H), 7.99 (s, 1 H), 7.93 (m, 1 H), 7.84 (m, 1 H,), 7.63 (m, 2 H), 5.40 (s, 1 H), 4.11 (m, 2 H), 3.16 (m, 2 H). MS *m/z* 301 (M^+), 273 (100), 186, 158, 142, 114. Anal. ($C_{15}H_{11}NO_2S_2$) C, H, N.

(±)-1,13a-Dihydro-3*H*,5*H*,11*H*-naphto[2,3-*f*]thiazolo[4,3-*c*][1,4] thiazepine-5,13-dione (20). Starting from 3,3'-dithiobis(2,2'-naphtoic acid) **(32f)** (1.22 g, 1.5 mmol), the compound **20** (0.34 g, 38% yield from the thiophenol precursor) was obtained, using the same procedure as for **10**, but carrying out the $NaBH_4$ reduction of the disulfide overnight at room temperature: mp 200-203 °C; IR (KBr) 1695, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.51 (s, 1 H), 7.98 (s, 1 H), 7.94 (m, 1 H), 7.84 (m, 1 H,), 7.58 (m, 2 H), 4.85 (AB q, 2 H, J = 10.6 Hz), 4.66 (dd, 1 H, J = 6.9, 1.6 Hz), 3.65 (dd, 1 H, J = 11.8, 1.5 Hz), 3.14 (dd, 1 H, J = 12.1, 6.7 Hz). MS *m/z* 301 (M^+), 273 (100), 186, 158, 142, 114. Anal. ($C_{15}H_{11}NO_2S_2$) C, H, N.

(±)-2,3,11,11a-Tetrahydro-11-hydroxy-11-phenyl-1*H*,5*H*-pyrrolo [2,1-*c*][1,4]benzothiazepin-5-one (26). A solution of compound **37**[16] (0.88 g, 3.8 mmol) in dry THF (10 mL) was dropwise added to a solution of PhMgBr in ethyl ether [obtained from 1.23 g of PhBr, 0.185 g of Mg turnings and 8 mL of Et_2O]. The mixture was refluxed for 30 min, then cooled to rt and quenched by the addition of NH_4Cl saturated solution. Chloroform extraction and evaporation gave a residue which was purified by column chromatography to give the title compound **26** (0.7 g, 59% yield) along with some other unidentified by-products. The title compound was obtained as colorless crystals by crystallization: mp 137-141°C (ethanol); IR (KBr) 3210 broad, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.02 (m,

1 H), 7.85 (m, 1 H), 7.60-7.10 (m, 7 H), 3.87 (m, 1 H), 3.55 (m, 2 H), 3.08 (br s, 1 H), 1.95 (m, 4 H). Anal. ($C_{18}H_{17}NO_2S$) C, H, N.

(±)-*cis*-2,3,11,11a-Tetrahydro-11-(4-methylpiperazin-1-yl)-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]benzothiazepin-5-one (27). A mixture of (+)-11-chloro-2,3,11,11a-tetrahydro-1*H*,5*H*,-pyrrolo[2,1-*c*][1,4]benzothiazepin-5-one **(38)**[15] (0.6 g, 2.4 mmol), freshly distilled 1-methylpiperazine (0.34 mL, 3.1 mmol), Na_2CO_3 (1.06 g, 10.0 mmol), NaI (0.36 g, 2.4 mmol) in dry CH_3CN (30 mL) was gently refluxed for 20 hours. The solvent was evaporated and the residue was partitioned between CH_2Cl_2 and water. The organic phase was successively washed with a 2% solution of $Na_2S_2O_3$, water and brine. The residue obtained after evaporation was chromatographed on silica gel eluting with 5% methanol in CH_2Cl_2. The title compound **27** was obtained as a white solid (0.55 g, 73% yield): mp 188-190°C (2-propanol - isopropyl ether); IR (KBr) 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.71 (m, 1 H), 7.50 (m, 2 H), 7.61 (m, 1 H), 7.33 (m, 2 H), 4.36 (d, 1 H, J = 11.2 Hz), 3.70 (m, 3 H), 2.58 (m, 4 H), 2.38 (m, 4 H), 2.27 (s, 3 H), 2.02 (m, 4 H). MS *m/z* 317 (100, M^+), 217, 180, 139, 99, 70. Anal. ($C_{17}H_{23}N_3OS$) C, H, N.

(±)-3-[(4-Methylpiperazin-1-yl)methyl]-11-phenyl-5*H*,11*H*-pyrrolo [2,1-*c*][1,4]benzothiazepine (31). A solution of compound **39** [20] (0.55 g, 2.0 mmol), paraformaldehyde (0.1 g) and 1-methylpiperazine dihydrochloride (0.52 g, 3.0 mmol) in CH_3OH (20 mL) was refluxed for 48 hours. After cooling, the mixture was diluted with water and made alkaline (pH 9-10) by dropwise addition of 1N NaOH. The oil formed was extracted with ethyl acetate. The organic layer was washed with water and dried. After evaporation of the solvent, the residue obtained was chromatographed (5% CH_3OH in CH_2Cl_2) to give the title compound **31** as a white solid (0.7 g, 91% yield): mp 161-162°C (2-propanol); ^1H NMR ($CDCl_3$) δ7.50-7.00 (m, 9 H), 5.97 (s, 1 H), 5.86 (d, 1 H, J = 3.5 Hz), 5.53 (d, 1 H, J = 3.5 Hz), 5.36 (AB q, 2 H, J = 14.5 Hz), 3.50 (AB q, 2 H, J = 13.5 Hz), 2.52 (m, 8 H), 2.30 (s, 3 H). Anal. ($C_{24}H_{27}N_3S$) C, H, N.

EXAMPLE 4

Procedure for the Preparation of Sulfoxides 15-18

This procedure is illustrated for the preparation of (±)-*cis*-1,11a-Dihydro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4]benzothiazepine-5,11-dione 2-oxide **(16)**. To a stirred and cooled (0°C) solution of compound **5** [15] (0.5 g, 2.0 mmol) in dry dichloromethane (5 mL) ~80% 3-chloroperbenzoic acid (0.43 g, ~ 2 mmol) in 8

mL of the same solvent was added dropwise over about 15 minutes. After an additional 2 hours at 0°C, the reaction mixture was filtered and the filter cake was rinsed with dichloromethane. The combined solution was washed twice with 5% aqueous K_2CO_3, dried, and evaporated to give the crude **16** (0.47 g, 89% yield), which solidified on trituration with hexane: mp 191-194°C (benzene); IR (KBr) 1695, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.04 (m, 1 H,), 7.55 (m, 3 H,), 5.75 (dd, 1 H, J = 13.1, 3.0 Hz), 5.14 (t, 1 H, J = 7.6 Hz), 3.91 (d, 1 H, J = 13.1 Hz), 3.72 (dd, 1 H, J = 14.6, 7.6 Hz), 3.23 (ddd, 1 H, J = 14.6, 7.6, 3.0 Hz). Anal. ($C_{11}H_9NO_3S_2$) C, H, N.

(±)-*cis*-2,3-Dihydro-7-methyl-5*H*-thiazolo[2,3-*c*][1,4]benzothiazepine-5,11 (11a*H*)-dione 1-Oxide (15). Starting from **4** (0.53 g, 2.0 mmol), the compound **15** (0.39 g, 73% yield) was obtained using an identical procedure as for **16**: mp 211-212°C (benzene); IR (KBr) 1690, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.79 (d, 1 H, J = 2.5 Hz), 7.38 (m, 2 H), 5.25 (s, 1 H), 4.52 (m, 1 H), 3.40 (m, 1 H), 3.10 (m, 1 H), 2.47 (t, 3 H). Anal. ($C_{12}H_{11}NO_3S_2$) C, H, N.

(±)-*cis*-7-Chloro-1,11a-dihydro-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione 2-Oxide (17). Starting from **6** (0.57 g, 2.0 mmol), the compound **17** (0.41 g, 68% yield) was obtained using an identical procedure as for **16**: mp 213-215°C (benzene); IR (KBr) 1695, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ8.04 (d, 1 H, J = 2.7 Hz), 7.50 (m, 2 H), 5.75 (dd, 1 H, J = 12.9, 2.9 Hz), 5.13 (t, 1 H, J = 7.8 Hz), 3.91 (d, 1 H, J = 13.0 Hz), 3.73 (dd, 1 H, J = 14.7, 7.2 Hz), 3.23 (ddd, 1 H, J = 14.7, 7.8, 2.9 Hz). Anal. ($C_{11}H_8ClNO_3S_2$) C, H, N.

(±)-*cis*-1,11a-Dihydro-7-methoxy-3*H*,5*H*,11*H*-thiazolo[4,3-*c*][1,4] benzothiazepine-5,11-dione 2-Oxide (18). Starting from **10** (0.56 g, 2.0 mmol), the compound **18** (0.42 g, 71%yield) was obtained using an identical procedure as for **16**: mp 172-174°C (benzene); IR (KBr) 1690, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ7.55 (d, 1 H, J = 2.7 Hz), 7.40 (d, 1 H, J = 8.3 Hz), 7.09 (dd, 1 H, J = 8.3, 2.7 Hz), 5.76 (dd, 1 H, J = 12.9, 2.8 Hz), 5.17 (t, 1 H, J = 7.6 Hz), 3.91 (d, 1 H, J = 12.9 Hz), 3.89 (s, 3 H), 3.73 (dd, 1 H, J = 14.7, 7.1 Hz), 3.23 (ddd, 1 H, J = 14.7, 7.6, 2.8 Hz). Anal. ($C_{12}H_{11}NO_4S_2$) C, H, N.

EXAMPLE 5

Integrase Assays

The HPLC purified oligonucleotides AE117 (5'-ACTGCTAGAGATTTTCCACAC-3'); AE118 (5'-GTGTGGAAAATCTCTAGCAGT-3'); AE157 (5'-GAAAGCGACCGCGCC-3'); AE146 (5'-GGACGCCATAGCCCCGGCGCGGTCGCTTTC-3'); AE156 (5'-GTGTGGAAAATCTCTAGCAGGGGCTATGGCGTCC-3'); RM22M (5'-TACTGCTAGAGATTTTCCACAC-3'); and RMAB2 (5'-GTGTGGAAAATCTCTAGCUGT-3') were purchased from Midland Certified Reagent Company (Midland, TX). An expression system for the wild-type integrase and the IN^{50-212} (F185K) were obtained from the Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD.

To analyze the extent of 3'-processing and strand transfer using 5'-end labeled substrates, AE118 was 5'-end labeled using T_4 polynucleotide kinase (Gibco BRL) and $\gamma[^{32}P]$-ATP (Dupont-NEN). To determine the extent of 30-mer target strand generation during disintegration, AE157 was 5'-end labeled and annealed to AE156, AE146, and AE117. The kinase was heat-inactivated and AE117 was added to the same final concentration. The mixture was heated at 95°C, allowed to cool slowly to room temperature, and run through a G-25 Sephadex quick spin column (Boehringer Mannheim, Indianapolis, IN) to separate annealed double-stranded oligonucleotide from unincorporated label.

To analyze the extent of site-specific cleavage of 3'-end-labeled substrate by integrase, AE118 was 3'-end-labeled using $\gamma[^{32}P]$-cordycepin triphosphate (Dupont-NEN) and terminal transferase (Boehringer Mannheim). The transferase was heat-inactivated, and RM22M was added to the same final concentration. The mixture was heated at 95°C, allowed to cool slowly to room temperature, and run through a G-25 spin column as before.

To determine the extent of Schiff base formation, RMAB2 was 5'-end labeled and reacted with AE117 as described above. The uracil was removed from duplex oligonucleotide containing deoxyuridine by incubation of 40 μl of end-labeled DNA (500 nM stock solution) with 1 unit of uracil DNA glycosylase (Life Technologies, Inc.) for 90 minutes at 30 C. The reaction was then loaded on

a G-25 Sephadex quick spin column to remove the unincorporated label and the uracil.

To determine the extent of 3'-processing and strand transfer, integrase was preincubated at a final concentration of 200 nM with the inhibitor in a reaction buffer (50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/ml bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM MOPS, pH 7.2) at 30°C for 30 minutes. Then, 20 nM of the 5'-end ^{32}P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional one hour. Reactions were quenched by the addition of an equal volume (16 μl) of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol and 0.025% bromophenol blue). An aliquot (5 μl) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M tris-borate pH 8.3, 2 mM EDTA, 20% acrylamide, 8M urea).

Gels were dried, exposed in a PhosphorImager cassette, and analyzed using a Molecular Dynamics PhosphorImager (Sunnyvale, CA). Percent inhibition was calculated using the following equation:

$$\%I = 100 \text{ X } [1 - (D - C)/(N - C)]$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3'-processing product) or strand transfer products for DNA alone (C), DNA plus integrase (N), and integrase plus drug (D). All IC_{50} values were determined by plotting the drug concentration versus percent inhibition, and determining the concentration which produced 50% inhibition.

To determine the effects of drugs on the choice of nucleophile in the 3'-processing, reactions were performed essentially as described above with a 3'-end labeled oligonucleotide. Disintegration reactions were performed as above with a Y oligonucleotide (i.e., the branched substrate in which the U5 end was "integrated" into target DNA).

EXAMPLE 6

HIV-1 Cell and Target-based Assays

The cell-based p24 attachment assay has been described in detail elsewhere.[33] Assays for activity against HIV-1 reverse transcriptase rAdT (template/primer) and rCdG (template/primer) using recombinant HIV-1 reverse transcriptase, have been previously described.[34] The substrate

cleavage of recombinant HIV-1 protease in the presence of test compounds was quantified using an HPLC-based methodology with the artificial substrate Ala-Ser-Glu-Asn-Try-Pro-Ile-Val-amide (Multiple Peptide Systems, San Diego, CA) as has been previously described.[33,35]

EXAMPLE 7

Anti-HIV Assays in Cultured Cell Lines

The anti-HIV drug testing was performed at NCI based on a protocol described by Weislow et al.[36] All compounds were dissolved in dimethyl sulfoxide and diluted 1:100 in cell culture medium. Exponentially growing T4 lymphocytes (CEM cell line) were added at 5000 cells per well. Frozen virus stock solutions were thawed immediately before use, suspended in complete medium to yield the desired multiplicity of infection (m. o. i. $\approx$ 0.1), and added to the microtiter wells, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls.

Cultures were incubated at 37°C in a 5% CO_2 atmosphere for 6 days. The tetrazolium salt, XTT was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

All positive control compounds for individual assays except AZTTP were obtained from the NCI chemical repository. The reference reagents for the individual assays were as follows:

attachment: Farmatalia (NSC 65016)[29] and dextran sulfate (NSC 620255);

reverse transcriptase inhibition: rAdT Template/primer-AZTEC (Sierra BioResearch, Tuscon, AZ), rCdG Template/primer-UC38[30] (NSC 629243);

protease inhibition: KNI-272[31] (NSC 651714).

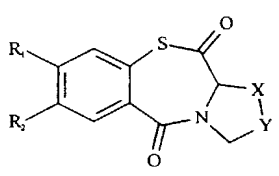
EXAMPLE 8

Identification of Anti-Integrase and Anti-Viral Compounds

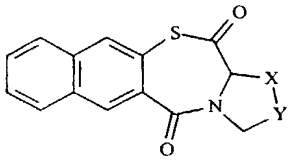
The thiazepines of the present invention were tested for anti-integrase and anti-viral activity, and the results are shown in Table 1.

Table 1

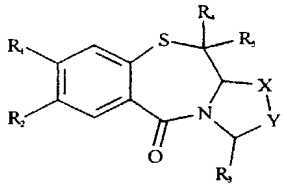
Anti-HIV-1 Integrase Activities of Thiazepines 1-31



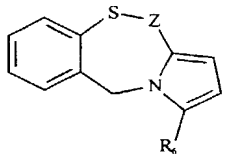
1-16



19-20



21-27



28-31

Compd	R_1	R_2	R_3	R_4	R_5	R_6	-X-Y-	Z	Integrase Assay IC_{50} (μM) 3'-Processing	3'-end joining	Cell data (μM) $^aEC_{60}$	$^bCC_{50}$
1	H	H					-S-CH_2-		110±12	146±79	107	>200
2	H	Cl					-S-CH_2-		151; 105	120; 60	>50	>50
3	H	Br					-S-CH_2-		58±15	48±18		
4	H	Me					-S-CH_2-		64±47	55±29		
5	H	H					-CH_2-S-		208±24	227±104	>200	>200
6	H	Cl					-CH_2-S-		158; 111	160; 110	>100	>100
7	H	Br					-CH_2-S-		87±24	74±32	>200	>200
8	H	Me					-CH_2-S-		52	63	NR	35
9	NO_2	H					-CH_2-S-		90±27	100±36	>50	>50
10	H	OMe					-CH_2-S-		155; 275	155; 245	>200	>200
11	OMe	OMe					-CH_2-S-		670; 630	333; 330	>50	>50
12	H	H					-CH_2-O-		>333	>333		
13	OMe	OMe					-$(CH_2)_2$-		>1000	>1000	>200	>200
14	H	H					-$(CH_2)_3$-		406; 495	343±109	>200	>200
15	H	Me					-S(O)-CH_2-		590±350	590±350	>100	>100
16	H	H					-CH_2-S(O)-		200; 185	215; 222		
17	H	Cl					-CH_2-S(O)-		260; 215	280; 200		
18	H	OMe					-CH_2-S(O)-		84.5	142		
19							-S-CH_2-		40±10	47±6	60	>316
20							-CH_2-S		92±30	100±40	280	>316
21	H	H	H	OAc	H		-$(CH_2)_2$-		>1000	>1000	>200	>200
22	H	H	H	OMe	H		-$(CH_2)_2$-		>1000	>1000	>200	>200
23	H	H	Ph	H	H		-CH_2-S-		372; 111	376; 288		
24	OMe	OMe	H	H	H		-$(CH_2)_2$-		>1000	>1000	>200	>200
25	H	H	H	OH	H		-$(CH_2)_2$-		>1000	>1000	>200	>200
26	H	H	H	OH	Ph		-$(CH_2)_2$-		>1000	>1000		
27	H	H	H	H	$N(CH_2CH_2)_2NMe$		-$(CH_2)_2$-		>1000	>1000	>125	>125
28						H		>CO	590	300	146	>200
29						H		>$CHCO_2Et$	>1000	>1000		
30						CH_2NMe_2		>CHC_6H_4-*p*F	>1000	>1000		
31						$CH_2N(CH_2CH_2)_2NMe$		>CHPh	>1000	>1000		

$^aEC_{60}$: 50% effective concentration
$^bCC_{50}$: 50% cytotoxic concentration

Benzothiazepine **1** was identified as an IN inhibitor (IC_{50} values for 3'-processing and 3'-end joining: 110 and 146 μM, respectively) by showing it to have antiviral activity in the NCI Antiviral Drug Screen against CEM cells (Example 7). Compound **1** with a therapeutic index ($TI=CC_{50}/EC_{50}$) value of > 1.8 (50% effective concentration [EC_{50}] value of 107±26 and a 50% cytotoxic concentration [CC_{50}] value of > 200 μM) is moderately active in HIV-1 infected

CEM cells. This compound was also found to be relatively non-cytotoxic, such that it would more specifically affect HIV than normal host cells. Hence once the inventors recognized the anti-integrase activity of this compound, benzothiazepine **1** served as a "lead" compound for the design and testing of more potent derivatives.

In order to establish a structure-activity relationship, 30 analogs were prepared and tested in an assay specific for IN as well as against HIV-1-infected CEM cells (see Table 1). Several modifications were made to determine the biological effect of each ring and the substituents. The first modifications were aimed at the A and B rings (compounds **1-18**). In a second class of compounds, an extra benzene ring was added, and the effect of the position of the sulfur in the thiazolo ring was analyzed (**19** and **20**). The third group contains substitutions on all the rings (**21-27**) and the last group (**28-31**) was designed to determine the effect of the thiazepine ring system on biological activity.

As illustrated in Table 1, the chloro-, bromo-, and the methyl-substituted derivatives **2-4**, exhibited potency similar to the parent compound **1** against purified IN. Compound **2** was tested for antiviral activity only at 50 μM and lower. Comparable results were obtained when sulfur was moved to position 10 as in compounds **5-11** (Table 1). However, the removal of sulfur (compounds **12-14**) substantially reduced anti-IN and antiviral activities. Oxidation of the sulfur atom also generally reduced potency (compounds **15-18**), but with compound **18** showing the best anti-IN activity. Both of the naphtho-derivatives **19** and **20** showed antiviral as well as anti-IN activity. Compound **19** with an IC_{50} value of 40 μM against IN was more active than the parent compound **1** against HIV-1 infected cells. Compound **19** with a therapeutic index (TI) value of > 5 was the best in this series (EC_{50}: 60 and CC_{50}: > 316 μM). Moreover, the naphthalene substituted compound **20** was active both against purified IN (IC_{50} values of 92 and 100 μM against 3'-processing and strand transfer, respectively) and HIV-1 infected cells (EC_{50}: 279-286 and IC_{50}: >316 μM). Other modifications of the ring system (compounds **21-31**) reduced both antiviral and anti-IN activities.

In particular embodiments, but without limiting the invention, an EC50 of 200 μM or less identifies a compound that is particularly suitable for further investigation. A CC_{50} as high as possible is desired, for example at least 100, 200, 300 or even 1000. A TI value of at least 1 (for example 1.5 or greater) also helps identify compounds for further investigation.

EXAMPLE 9

Role of divalent metals

Divalent metal ions, such as Mn^{+2} or Mg^{+2}, coordinate with the acidic residues (D, D, E) of IN's active site.[27] Metals are involved in the catalytic functioning because the enzyme is unable to perform 3' processing and strand transfer without Mn^{+2} or Mg^{+2}. Previous studies have implied that hydroxylated aromatic inhibitors of IN are potentially metal chelators.[8,9,28] Thus, chelating metals at the active site of IN has been proposed to be responsible for the inhibition of IN function. However, hydroxylated aromatics are generally active only when Mn^{+2} is used as a cofactor.[8]

In contrast, the thiazolothiazepines of the present invention are equally active in Mg^{+2}-based assays. Figure 1 shows a representative gel comparing the inhibition of IN in the presence of Mn^{+2} or Mg^{+2}. A 21-mer oligonucleotide corresponding to the last 21 bases on the HIV-1 viral DNA in the U5 region of long terminal repeat (LTR) was radiolabeled with ^{32}P. This is shown in the Figs. 2 and 3 as a band corresponding to DNA alone. Integrase will cleave the last two bases of this DNA in the presence of Mn^{+2} or Mg^{+2} to give a 19-mer DNA substrate (lane 2) which runs faster on the gel and is indicated as 19-mer on the Figures. When an inhibitor is added this process is blocked, therefore the original 21-mer DNA would not be cut. When an inhibitor is added at various concentrations, percent inhibition at every concentration will be calculated by quantification of the intensity of each band using an Imagequant program.

The gels are run using polyacrylamide according to the standard procedures. Upon completion of electrophoresis run, the gels are dried and exposed to a phosphorimager cassette. The cassettes are analyzed by the phosphorimager, and the bands are shown on a computer screen. The intensity of each band is quantitated, plotted against concentrations of drugs, and the IC_{50} values are calculated from the graphs.

Compounds **1, 19** and **20** were active in the presence of Mg^{+2} within the same concentration range as in Mn^{+2}, thus indicating that these compounds differ from hydroxylated aromatics and perhaps act at different sites on IN. Inhibition of IN in the presence of Mg^{+2} by thiazolothiazepines sets this class of compounds apart from other IN inhibitors. The activity in Mg^{+2} is believed to be related to the antiviral activity of the thiazolothiazepines and derivatives, because Mg^{+2} has been proposed to be the metal used *in vivo* by IN. Hence compounds having activity that is inhibited in the presence of Mg^{2+} would be expected to have less antiviral activity in the presence of the viral Mg^{2+}.

EXAMPLE 10

Selectivity of Compounds

The selectivity of compounds **1, 19,** and **20** was examined against other sites on the HIV replication cycle. Assays for activity against HIV-1 reverse transcriptase rAdT (template/primer) and rCdG (template/primer) using recombinant HIV-1 reverse transciptase (from ABL Basic Research NCI-FCRDC, Frederick, MD) have been previously described.[30] A cell-based viral p24 attachment assay, and a nucleoprotein zinc finger assay, have also been described in detail in Rice et al.[33] The substrate cleavage of recombinant HIV-1 protease in the presence of test compounds was quantified using an HPLC-based methodology with the artificial substrate Ala-Ser-Glu-Asn-Tyr-Pro-Ile-Val-amide (Multiple Peptide Systems, San Diege, CA) as previously described.[30]

When tested against reverse transcriptase, protease, virus attachment, or nucleocapsid protein zinc fingers, none of the compounds exhibited any detectable activities at 100 μM, indicating moderate selectivity against IN. Thus, thiazolothiazepines are a group of selective inhibitors of HIV IN, that can serve as therapeutic compounds or lead compounds in the development of analogs and other derivatives.

The fact that thiazolothiazepines are equally potent in Mg^{+2}- and Mn^{+2}-based assays indicates that the IN binding site of these compounds differs from the binding site of previously reported inhibitors. Testing these compounds against other viral proteins (reverse transcriptase, protease, virus attachment, or nucleocapsid zinc fingers) shows selectivity of the compounds for IN, as compared to other steps in the retroviral life cycle. Another advantage of this class of compounds is that they are amenable for preparation of chemical libraries using recent advances in combinatorial chemistry, and can be used as novel lead compounds for designing drugs against IN and HIV replication.

Reference Reagents for Mechanistic and Target-based Assays. All positive control compounds for individual assays except AZTTP were obtained from the NCI chemical repository. The reference reagents for the individual assays are as follows:

attachment: Farmitalia (NSC 65016) and dextran sulfate (NSC 620255);

reverse transcriptase inhibition: rAdT Template/primer-AZTEC (Sierra BioResearch, Tuscon, AZ), rCdG Template/primer-UC38 (NSC 629243);

protease inhibition: KNI-272 (NSC 651714);

IN inhibitor: ISIS 5320 (NSC 665353) and DIBA-1 (NSC 654077) a NCp7 Zn finger inhibitor.

EXAMPLE 11

Methods of Treatment

The present invention includes a treatment for HIV disease and associated diseases, in a subject such as an animal, for example a rat or human. The method includes administering the compound of the present invention, or a combination of the compound and one or more other pharmaceutical agents, to the subject in a pharmaceutically compatible carrier and in an amount effective to inhibit the development or progression of HIV disease. Although the treatment can be used prophylactically in any patient in a demographic group at significant risk for such diseases, subjects can also be selected using more specific criteria, such as a definitive diagnosis of the condition.

The vehicle in which the drug is delivered can include pharmaceutically acceptable compositions of the drugs, using methods well known to those with skill in the art. Any of the common carriers, such as sterile saline or glucose solution, can be utilized with the drugs provided by the invention. Routes of administration include but are not limited to oral and parenteral rountes, such as intravenous (iv), intraperitoneal (ip), rectal, topical, ophthalmic, nasal, and transdermal.

The drugs may be administered intravenously in any conventional medium for intravenous injection, such as an aqueous saline medium, or in blood plasma medium. The medium may also contain conventional pharmaceutical adjunct materials such as, for example, pharmaceutically acceptable salts to adjust the osmotic pressure, lipid carriers such as cyclodextrins, proteins such as serum albumin, hydrophilic agents such as methyl cellulose, detergents, buffers, preservatives and the like. A more complete explanation of parenteral pharmaceutical carriers can be found in *Remington: The Science and Practice of Pharmacy* (19th Edition, 1995) in chapter 95.

Embodiments of other pharmaceutical compositions can be prepared with conventional pharmaceutically acceptable carriers, adjuvants and counterions as would be known to those of skill in the art. The compositions are preferably in the form of a unit dose in solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions.

The compounds of the present invention are ideally administered as soon as possible after potential or actual exposure to HIV infection. For example, once

HIV infection has been confirmed by laboratory tests, a therapeutically effective amount of the drug is administered. The dose can be given by frequent bolus administration.

Therapeutically effective doses of the compounds of the present invention can be determined by one of skill in the art, with a goal of achieving tissue concentrations that are at least as high as the IC_{50} of each drug tested in the foregoing examples. The low toxicity of the compound makes it possible to administer high doses, for example 100 mg/kg, although doses of 10 mg/kg, 20 mg/kg, 30 mg/kg or more are contemplated. An example of such a dosage range is 0.1 to 200 mg/kg body weight orally in single or divided doses. Another example of a dosage range is 1.0 to 100 mg/kg body weight orally in single or divided doses. For oral administration, the compositions are, for example, provided in the form of a tablet containing 1.0 to 1000 mg of the active ingredient, particularly 1, 5, 10, 15, 20, 25, 50, 100, 200, 400, 500, 600, and 1000 mg of the active ingredient for the symptomatic adjustment of the dosage to the subject being treated.

The specific dose level and frequency of dosage for any particular subject may be varied and will depend upon a variety of factors, including the activity of the specific compound, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, and severity of the condition of the host undergoing therapy.

The pharmaceutical compositions can be used in the treatment of a variety of retroviral diseases caused by infection with retroviruses that require integrase activity for infection and viral replication. Examples of such diseases include HIV-1, HIV-2, the simian immunodeficiency virus (SIV), the feline immunodeficiency virus (FIV), HTLV-1, HTLV-2, spumavirus (human foamy virus) and feline infectious leukemia.

EXAMPLE 12

Combination Therapy

The present invention also includes combinations of HIV integrase inhibitor compounds with one or more agents useful in the treatment of HIV disease. For example, the compounds of this invention may be administered, whether before or after exposure to the virus, in combination with effective doses of other anti-virals, immunomodulators, anti-infectives, or vaccines. The term

"administration" refers to both concurrent and sequential administration of the active agents.

Example of antivirals that can be used in combination with the integrase inhibitors of the invention are: AL-721 (from Ethigen of Los Angeles, CA), recombinant human interferon beta (from Triton Biosciences of Alameda, CA), Acemannan (from Carrington Labs of Irving, TX), gangiclovir (from Syntex of Palo alto, CA), didehydrodeoxythymidine or d4T (from Bristol-Myers-Squibb), EL10 (from Elan Corp. of Gainesville, GA), dideoxycytidine or ddC (from Hoffman-LaRoche), Novapren (from Novaferon labs, Inc. of Akron, OH), zidovudine or AZT (from Burroughs Wellcome), ribaririn (from Viratek of Costa Mesa, CA), alpha interferon and acyclovir (from Burroughs Wellcome), Indinavir (from Merck & Co.), 3TC (from Glaxo Wellcome), Ritonavir (from Abbott), Saquinavir (from Hoffmann-LaRoche), and others.

Examples of immuno-modulators that can be used in combination with the integrase inhibitors of the invention are AS-101 (Wyeth-Ayerst Labs.), bropirimine (Upjohn), gamma interferon (Genentech), GM-CSF (Genetics Institute), IL-2 (Cetus or Hoffman-LaRoche), human immune globulin (Cutter Biological), IMREG (from Imreg of New Orleans, La.), SK&F106528, and TNF (Genentech).

Examples of some anti-infectives with which the integrase inhibitors can be used include clindamycin with primaquine (from Upjohn, for the treatment of pneumocystis pneumonia), fluconazlone (from Pfizer for the treatment of cryptococcal meningitis or candidiasis), nystatin, pentamidine, trimethaprim-sulfamethoxazole, and many others.

The combination therapies are of course not limited to the lists provided in these examples, but includes any composition for the treatment of HIV disease (including treatment of AIDS).

EXAMPLE 13

Obtaining HIV Integrase Inhibitors by Combinatorial Chemistry

Combinatorial chemistry allows generation of large numbers of unique molecules with a small number of chemical reactions. Rather than using the traditional approach of synthesizing novel compounds one at a time, compounds are synthesized by performing chemical reactions in stages, and reacting all of the molecules formed in stage n-1 with each reactant in stage n. Such techniques can be used to obtain analogs and other variants of the thiazepines of the present invention, and to test them for anti-integrase activity.

In view of the many possible embodiments to which the principles of the invention may be applied, it should be recognized that the illustrated embodiments are only particular examples of the invention and should not be taken as a limitation on the scope of the invention. Rather, the scope of the invention is defined by the following claims. We therefore claim as our invention all that comes within the scope and spirit of these claims.

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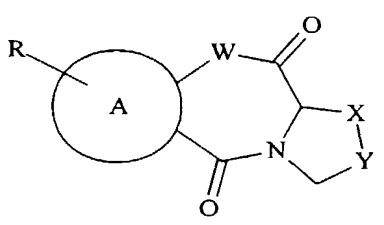
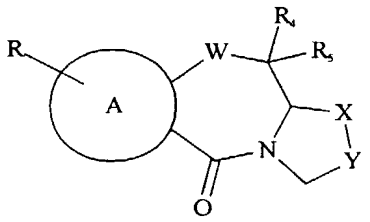
We claim:

1. A method of inhibiting a HIV integrase, the method comprising:

exposing the integrase to an integrase inhibiting amount of one or more anti-integrase compounds selected from the group consisting of the following compounds, or pharmaceutically acceptable salts thereof:





wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline;

R is one or more of H, halogen, lower alkyl, lower alkoxy, NO_2, lower ester or carboxylic acid;

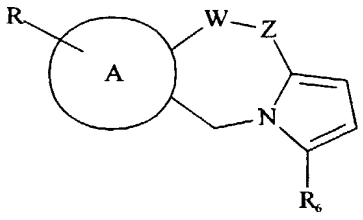
X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2;

R_4 is H or hydroxy;

R_5 is H, phenyl, or alkylamine; and

W is S or O.

or wherein the compound is



wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; and

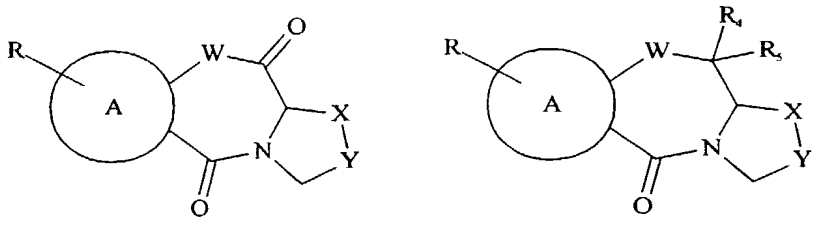
R is one or more of H, halogen, lower alkyl, lower ester or carboxylic acid;

R_6 is H, substituted or unsubstituted alkyl or amine;

W is S or O; and

Z is S, O, CH_2, CH_2CH_2, or C=O.

2. The method of claim 1, wherein the compound is selected from the group consisting of:



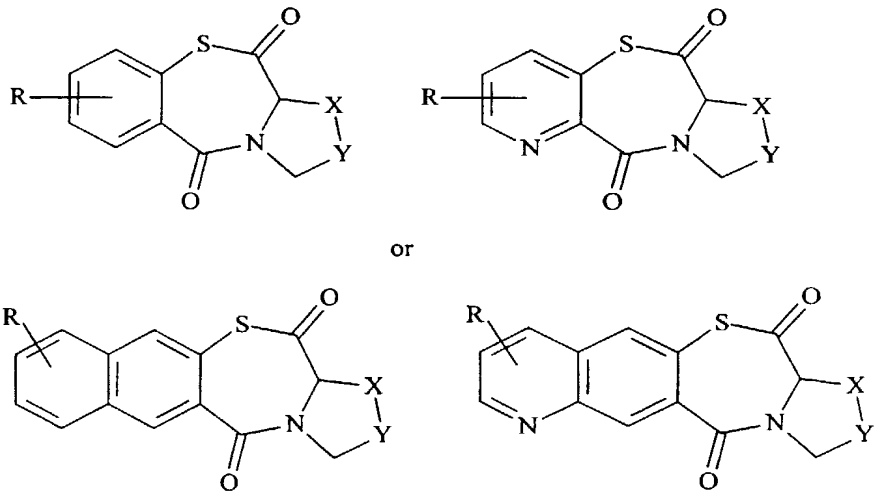
wherein X-Y is CH_2-S, S-CH_2, CH_2-O, or CH_2-CH_2, and W is S.

3. The method of claim 2, wherein:
A is benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline.

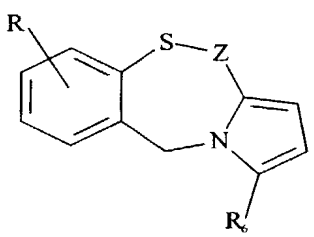
4. The method of claim 3, wherein A is benzene or naphthalene.

5. The method of claim 4, wherein R is H, halogen, lower alkoxy, or NO_2.

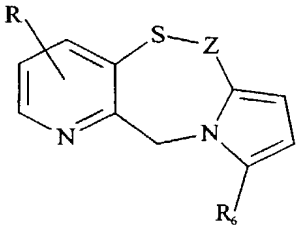
6. The method of claim 1, wherein the compound is:



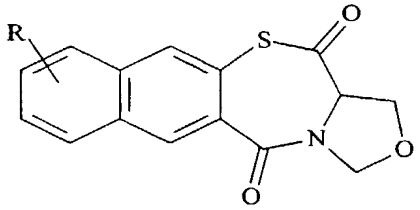
7. The method of claim 1, wherein the compound is:



or



8. The method of claim 6, wherein the compound is



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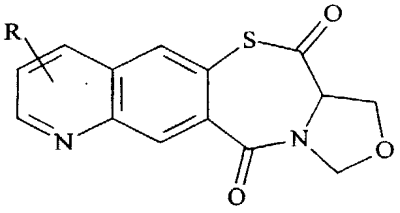
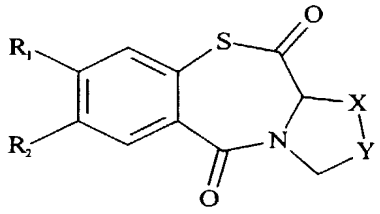
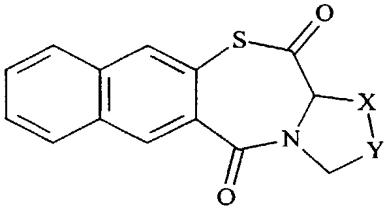
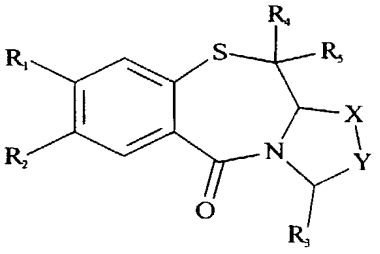


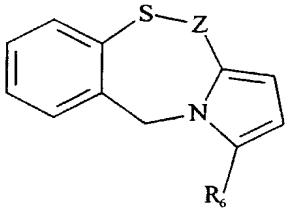
9. The method of claim 1, wherein the compound is one of the following:







or



wherein X-Y is S-CH_2, CH_2-S, CH_2-O, CH_2-CH_2, S(O)-CH_2, or CH_2-S(O);

R_1 and R_2 are independently selected from the group consisting of H, NO_2, halogen, lower alkyl or lower alkoxy;

R_3 is H or phenyl;

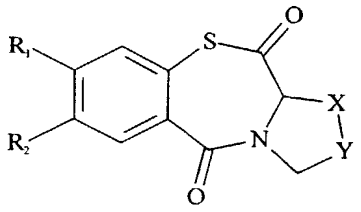
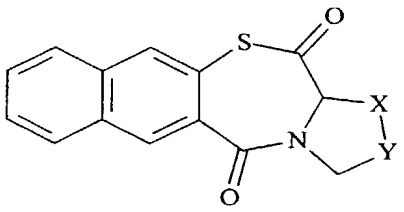
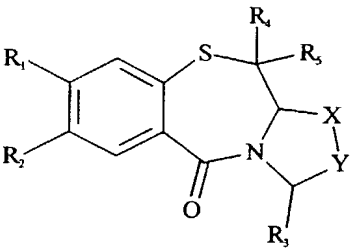
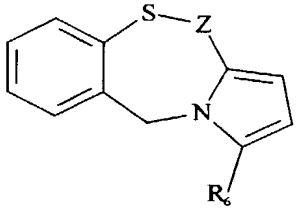
R_4 is H or hydroxy;

R_5 is H, phenyl or alkylamine; and

R_6 is H, phenyl or alkylamine.

10. The method of claim 9, wherein the alkylamine is $-N(CH_2CH_2)_2NCH_3$,$-CH_2NCH_2CH_3$, or $-CH_2N(CH_2CH_2)_2NCH_3$.

11. The method of claim 7, wherein the compound is




or


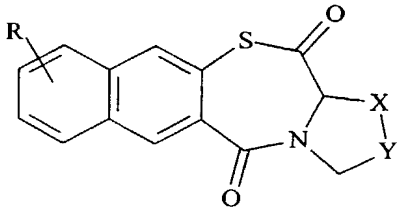
X-Y is $S-CH_2$, CH_2-S, or $CH_2-S(O)$;

and R_1 and R_2 are independently selected from the group consisting of H, NO_2, halogen, lower alkyl and lower alkoxy;

R_3 is H; and

R_4, R_5, and R_6 are H.

12. The method of claim 1, wherein the compound is



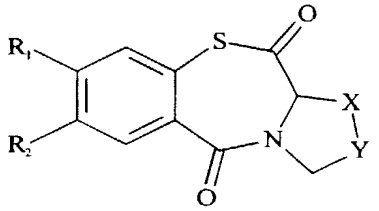
and X-Y is $S-CH_2$ or CH_2-S.

13. The method of claim 12, wherein R is H.

14. The method of claim 13, wherein X-Y is $S-CH_2$.

15. The method of claim 9, wherein
R_1 is H, NO_2, or lower alkoxy,
R_2 is H, Cl, Br, lower alkyl, or lower alkoxy;
R_3 and R_4 are H;
R_5 is $N(CH_2CH_2)_2NCH_3$; and
X-Y is CH_2-S, S-CH_2, or CH_2-CH_2.

16. The method of claim 15 wherein the compound is



wherein R_1 is H, NO_2, or methoxy;
R_2 is H, halogen or methoxy; and
X-Y is CH_2-S or S-CH_2.

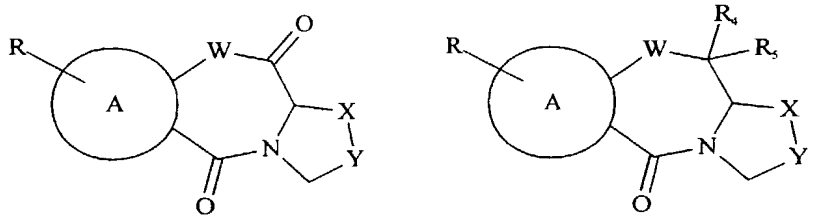
17. The method of claim 1, wherein the compound is administered in a therapeutically effective amount to a subject.

18. The method of claim 17, wherein the method is a method of treating or preventing HIV infection in the subject.

19. The method of claim 15, wherein the compound is administered in a therapeutically effective amount to a subject to treat or prevent an HIV infection.

20. The method of claim 16, wherein the compound is administered in a therapeutically effective amount to a subject to treat or prevent an HIV infection.

21. A method of treating or preventing HIV infection in a subject, comprising administering to the subject a therapeutically effective amount of a compound selected from the group consisting of:



wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline;

R is one or more of H, halogen, lower alkyl, lower alkoxy, NO_2, lower ester or carboxylic acid;

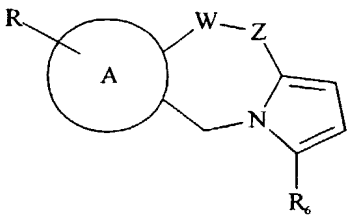
X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2;

R_4 is H or hydroxy;

R_5 is H, phenyl, or alkylamine; and

W is S or O.

or wherein the compound is



wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; and

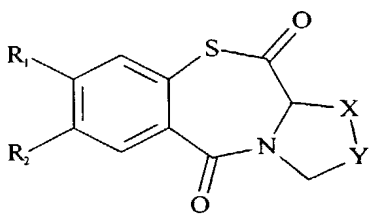
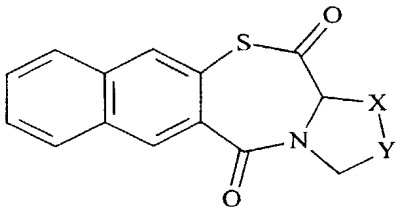
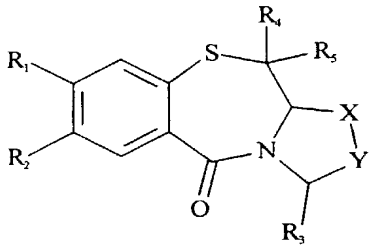
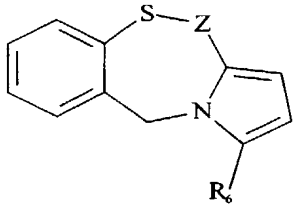
R is selected from the group of H, halogen, lower alkyl, lower ester or carboxylic acid;

R_6 is H, substituted or unsubstituted alkyl or amine;

W is S or O; and

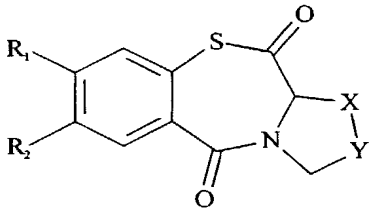
Z is S, O, CH_2, CH_2CH_2, or C=O.

22. The method of claim 21, wherein the compound is selected from the group consisting of:




and


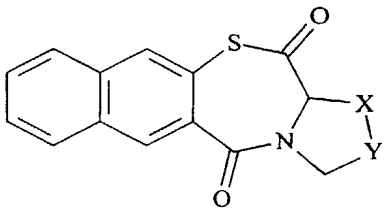
wherein X-Y is S-CH_2, CH_2-S, CH_2CH_2 or S(O)CH_2;
R_1 is H, NO_2, or lower alkoxy;
R_2 is H, Cl, Br, lower alkyl, or lower alkoxy;
R_3 and R_4 are H;
R_5 is $N(CH_2CH_2)_2NCH_3$; and
R_6 is H.

23. The method of claim 21, wherein the compound is



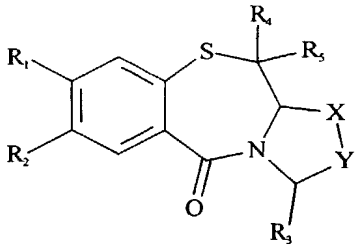
and
R_1 and R_2 are H, and X-Y is S-CH_2; or
R_1 is H, R_2 is Cl or Br or methyl, and X-Y is S-CH_2; or
R_1 is NO_2, R_2 is H, and X-Y is CH_2-S; or
R_1 and R_2 are methoxy, and X-Y is CH_2-S; or
R_1 is H, R_2 is methyl, and X-Y is S(O)-CH_2.

24. The method of claim 21, wherein the compound is



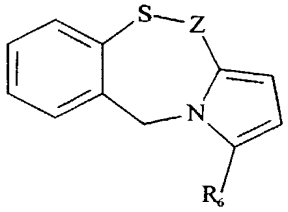
wherein X-Y is S-CH_2 or CH_2-S.

25. The method of claim 21, wherein the compound is



wherein X-Y is CH_2-CH_2;
R_1, R_2, R_3 and R_4 are H; and
R_5 is $N(CH_2CH_2)_2NCH_3$.

26. The method of claim 21, wherein the compound comprises



wherein R_6 is H and Z is C=O.

27. The method of claim 1, wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline;

R is one or more of halogen or NO_2;

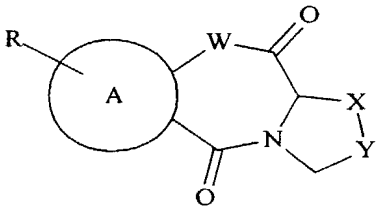
X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2;

R_4 is H or hydroxy;

R_5 is H, phenyl, or alkylamine;

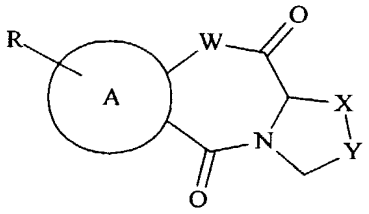
R_6 is H, or substituted or unsubstituted alkyl or amine; and
W is S or O.

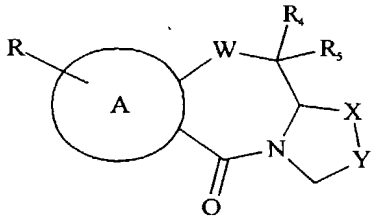
28. The method of claim 21, wherein the compound comprises



and A is benzene or naphthalene;
R is H, NO_2, or lower alkoxy; and
X-Y is CH_2-S or S-CH_2.

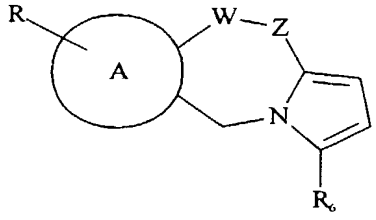
29. A compound having the following formula, or a pharmaceutically acceptable salt thereof:





wherein A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline;
R is one or more of halogen or NO_2;
X-Y is CH_2-S, S-CH_2, CH_2-O, CH_2-S(O), S(O)-CH_2, CH_2-CH_2, CH_2-CH_2-CH_2, or CH_2-CH_2-CH_2-CH_2;
R_4 is H or hydroxy;
R_5 is H, phenyl, or alkylamine; and
W is S or O.

or wherein the compound is



wherein

A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; and

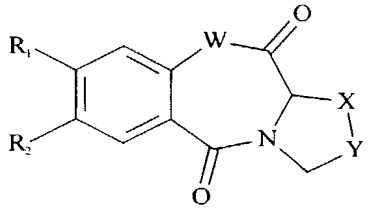
R is one or more of halogen or NO_2;

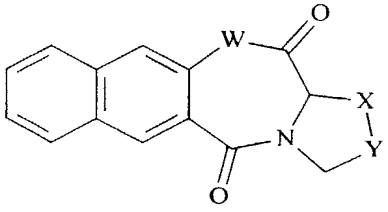
R_6 is H, substituted or unsubstituted alkyl or amine;

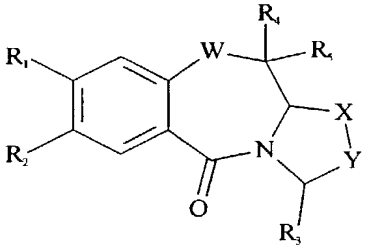
W is S or O; and

Z is S, O, CH_2, CH_2CH_2, or C=O.

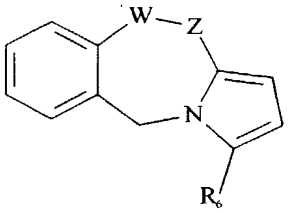
30. A compound having the following formula, or a pharmaceutically acceptable salt thereof, wherein the compound is:







or



wherein

X-Y is S-CH_2, CH_2-S, S(O)-CH_2, CH_2-S(O), or CH_2CH_2;

W is S or O;

R_1 is H or NO_2;

R_2 is H, halogen, lower alkyl or lower alkoxy;

R_3 is H;

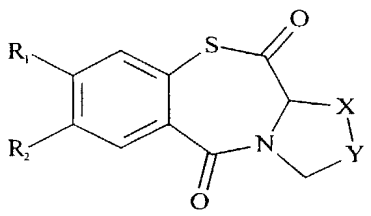
R_4 is hydroxy or H;

R_5 is phenyl or $N(CH_2CH_2)_2NCH_3$; and

R_6 is $CH_2N(CH_2CH_2)_2NCH_3$,

provided that R_1 and R_2 are not both H or not both alkoxy.

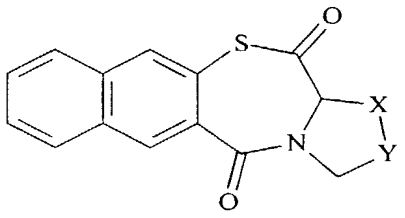
31. The compound of claim 30, wherein the compound is



and R_1 is H or NO_2;
R_2 is H, halogen, lower alkyl or lower alkoxy;
provided that R_1 and R_2 are not both H or not both alkoxy.

32. The compound of claim 30, wherein
R_1 is H, R_2 is Cl, X-Y is S-CH_2; or
R_1 is H, R_2 is Br, X-Y is S-CH_2; or
R_1 is H, R_2 is CH_3, X-Y is S-CH_2; or
R_1 is H, R_2 is H, X-Y is CH_2-S; or
R_1 is H, R_2 is Cl, X-Y is CH_2-S; or
R_1 is H, R_2 is Br, X-Y is CH_2-S; or
R_1 is H, R_2 is CH_3, X-Y is CH_2-S; or
R_1 is NO_2, R_2 is H, X-Y is CH_2-S; or
R_1 is H, R_2 is OCH_3, X-Y is CH_2-S; or
R_1 is H, R_2 is H, X-Y is CH_2-O; or
R_1 is H, R_2 is CH_3, X-Y is S(O)-CH_2; or
R_1 is H, R_2 is H, X-Y is CH_2-S(O); or
R_1 is H, R_2 is Cl, X-Y is CH_2-S(O); or
R_1 is H, R_2 is OCH_3, X-Y is CH_2-S(O).

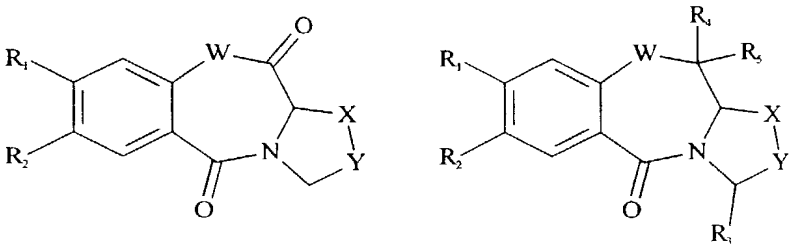
33. The compound of claim 30, wherein the compound is



and X-Y is S-CH_2 or CH_2-S.

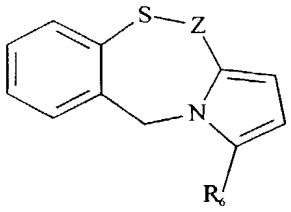
34. The compound of claim 30, wherein X-Y is S-CH_2.

35. The compound of claim 30, wherein the compound is:



and R_1, R_2 and R_3 are H, R_4 is OH or H;
R_5 is Ph or $N(CH_2CH_2)_2CH_3$; and
X-Y is CH_2-CH_2.

36. The compound of claim 30, wherein the compound is



and R_6 is $CH_2N(CH_2CH_2)_2NCH_3$.

37. A pharmaceutical composition comprising the compound of claim 29, or the pharmaceutically acceptable salt, and a pharmaceutically acceptable carrier.

38. A pharmaceutical composition comprising the compound of claim 30, or the pharmaceutically acceptable salt, and a pharmaceutically acceptable carrier.

39. A method of screening for an anti-HIV integrase drug, comprising:
providing an assay of HIV integrase inhibition; and
using the assay to screen for drugs comprising analogs or derivatives of any of the compounds of claim 1.

40. The method of claim 39, wherein the assay detects a thiazepine compound that inhibits human immunodeficiency virus type-1 intregrase (HIV-1 IN).

41. The method of claim 40, further comprising detecting a thiazepine having no detectable effect on reverse transcriptase, protease, and virus attachment.

42. The method of claim 39, wherein the compound is a thiazolothiazepine.

43. The compound of claim 29, for use in a pharmaceutical composition for the inhibition of HIV integrase.

44. The compound of claim 43, for use in the treatment of HIV infection.

45. The compound of claim 44, for use as a prophylactic treatment against HIV infection.

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07D 513/04, A61K 31/55, A61P 31/18 | A1

(11) International Publication Number: WO 00/68235

(43) International Publication Date: 16 November 2000 (16.11.00)

(21) International Application Number: PCT/US00/12847

(22) International Filing Date: 10 May 2000 (10.05.00)

(30) Priority Data:
60/133,726 12 May 1999 (12.05.99) US

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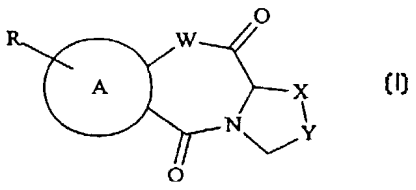
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG)

Published

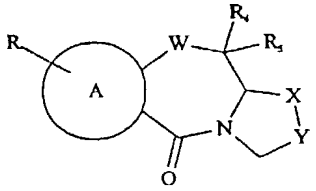
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

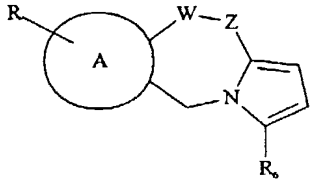
(54) Title: THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE



(I)



(II)



(III)

(57) Abstract

The present invention discloses non-catechol compounds, such as thiazolothiazepines, and analogs and derivatives thereof, which are anti-integrase inhibitors. The compounds, which are useful as treatments for HIV disease, include compounds (I), (II), (III), or pharmaceutically acceptable salts thereof wherein A is thiazole, benzene, naphthalene, pyridine, pyrimidine, pyrazine, or quinoline; R is one or more of H, halogen, lower alkyl, lower alkoxy, NO_2, lower ester or carboxylic acid; X–Y is CH_2–S, S–CH_2, CH_2–O, CH_2–S(O), S(O)–CH_2, CH_2–CH_2, CH_2–CH_2–CH_2, or CH_2–CH_2–CH_2–CH_2; R_4 is H or hydroxy; R_5 is H, phenyl, or alkylamine; W is S or O; and R_6 is H, substituted or unsubstituted alkyl or amine; and Z is S, O, CH_2, CH_2CH_2, or C=O.

FIG. 1

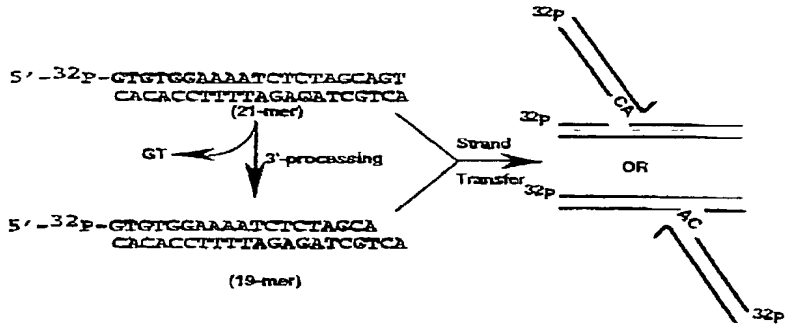


FIG. 2

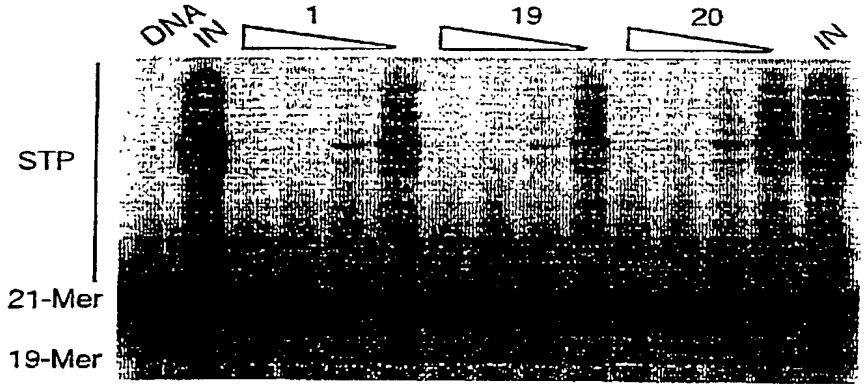
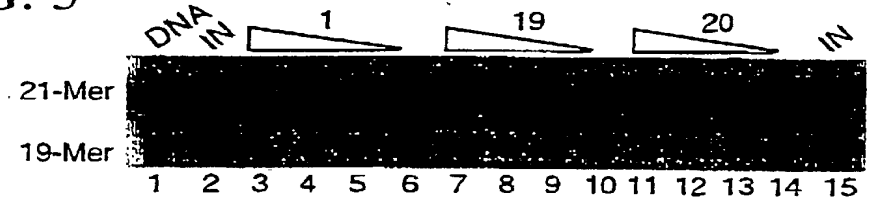


FIG. 3



WDN/:jam 4239-61380 E-036-99/1 10/17/01

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled THIAZEPINE INHIBITORS OF HIV-1 INTEGRASE , the specification of which

☒ is attached hereto.

☐ was filed on _____ as United States Application No. _____.

☐ was filed on _____ as International Application No. _____.

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of an PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
PCT/US00/12847	PCT	10 May 2000	☒ Yes ☐ No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

60/133,726	12 May 1999
(Application No.)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

WDN:Jan 4239-61380 E-036-99/1 11/07/01

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled, the specification of which

☒ is attached hereto.

☐ was filed on _____ as United States Application No. _____.

☐ was described and claimed in PCT International Application No. , filed on , and as amended under PCT Article 19 on (if applicable).

☐ and was amended on _____ (if applicable).

☐ with amendments through _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56. If this is a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, I further acknowledge the duty to disclose material information as defined in 37 CFR § 1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of an PCT International application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)	Country	Filing Date	Priority Claimed
PCT/US00/12847	PCT	10 May 2000	☒ Yes ☐ No

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WDN:jam 4230-61380 E-036-99/1 11/07/01

(Application No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application, to file a corresponding international application, and to transact all business in the Patent and Trademark Office connected therewith:

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25

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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WDN:jam 4239-61380 E-036-99/4 11/07/01

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